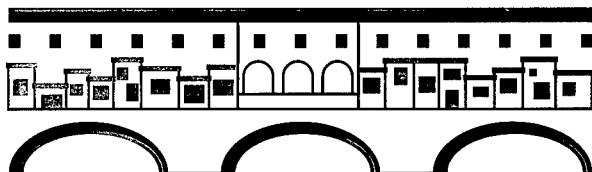*(handwritten) CSP 94-1016 (Partial)*

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METAL IONS IN
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EUROBIC II

Florence - Italy
August 30 - September 3, 1994

ABSTRACTS

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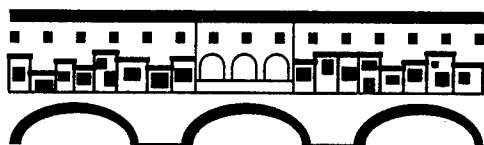Prescribed by ANSI Std. 239-18
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METAL IONS IN BIOLOGICAL SYSTEMS

EUROBIC II

Florence, Palaffari, August 30 - September 3, 1994

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Ilaria Baccetti and Irene Lozzi have helped with the organization

FOREWORD

This volume gathers the abstracts of the scientific contributions to the 2nd EUROBIC Conference. EUROBIC stands for European Bioinorganic Chemistry. Bioinorganic Chemistry is a growing field which is now well established at the global level. There is an International Conference on Bioinorganic Chemistry (ICBIC) which is held every odd year, an European Bioinorganic Chemistry Conference to be held every even year and in the Far East there is a tradition of meetings on Applied Bioinorganic Chemistry. Furthermore, to my knowledge, there is an annual Gordon Conference on Metal Ions in Biology and a biennial European Conference on the Chemistry of Metals in Biological Systems.

The European scientific community of Bioinorganic Chemistry is now trying to stick together even more, and to seek for more scientific visibility: there is a program of the European Science Foundation on the Chemistry of Metals in Biological Systems, it is the intention of many of us to give rise to an European Association, and there is the institution of the EUROBIC medal to be delivered to one European scientist.

Some of the people involved in this big venture are designated in this volume in the various committee listings. The medal has been designed by R. J. P. Williams. Most importantly, the medal is going to be delivered to W. Hagen for his scientific achievements and his distinguished personality. The motivation is in this volume.

Finally, it is a pleasure for me to thank Lucia Banci and Maria Silvia Viezzoli for fantastic scientific organization and for providing an appealing frame to the conference.

Ivano Bertini
Chairperson of the
Steering Committee

Profile of Research Achievements of the
European Bioinorganic Chemistry Medal Winner 1994
Professor W.R. Hagen

Professor W.R. (Fred) Hagen (born 1951, Amsterdam) did his PhD work with E.C. Slater and S.P.J. Albracht at the B.C.P. Jansen Institute of the University of Amsterdam. His thesis (1982) described EPR spectroscopic studies of the metal centres in mitochondrial and bacterial respiratory chain complexes. In the course of this work he became interested in the theoretical background of biological EPR, and this proved a major 'Leitmotif' in the ensuing years when he held a post-doc position (1982-1984) with W.R. Dunham and R.H. Sands at the Biophysics Research Division of the University of Michigan in Ann Arbor, and subsequently when he joined C. Veeger in the Biochemistry Department in Wageningen (1984-present).

While still in Amsterdam he developed an early version of what later became known as the statistical theory of g-strain: a description of inhomogeneously broadened powder EPR of transition metal complexes. This early theory, although mainly based on intuitive arguments, for the first time successfully reproduced the details of powder patterns from haem and iron-sulphur proteins and also made some qualitative predictions about anisotropy in the spin-lattice relaxation rates of these systems. The work was put on a firm theoretical basis when Hagen went in 1982 on an EMBO post doc to R.H. Sands and W.R. Dunham in Ann Arbor. A synthesis of statistical work by this group with Hagen's early work eventually led to a mathematically rigorous and generally applicable theory of g-strain culminating in a classical series of papers in the Journal of Magnetic Resonance. The theory has subsequently been applied to a variety of problems, *e.g.*, xanthine oxidase (with R. Hille), nitrogenase (with H. Haaker), ferredoxins and HiPIPs (with Dunham and Sands). At present, a collaborative effort with A.V. Xavier's group (Lisbon) is in progress on g-strain of multi-haem cytochromes.

Integer spin paramagnetism is another research project that started in Amsterdam and continues up to this time. Although integer spins are quite common in biology (*e.g.* ferrous proteins) many workers had long considered them to be undetectable by EPR. In 1982 Hagen applied parallel-mode EPR to powder samples, successfully predicted spectral shapes, and showed the applicability of the method to metalloproteins. This work was subsequently extended in Ann Arbor in a collaboration with Dunham when rigorous expressions were derived for the energy and transition probabilities of S=2 systems. This approach was then applied with H. Beinert (1984) to cytochrome oxidase. The first S=2 EPR from an iron-sulphur protein was also found in a collaboration with J.A. Fee and M.K. Johnson (1985). It took several years for the value of this approach to be appreciated by other groups and only recently has it become an active area of research worldwide. At present (1993) Hagen is

developing rigorous methods for the analysis of S=3 and S=4 systems present in several important enzymes.

Hagen's interest in iron-sulphur superclusters (clusters with Fe content higher than four) was stimulated in 1983 when D. Coucouvanis asked him to analyse the recently synthesised prismane model compounds using EPR. The previously developed g-strain software then proved essential to the identification of the highly anisotropic g-tensor characteristic of these 6Fe clusters. Three years later (1986) after returning to Wageningen, Hagen obtained the first indications, from EPR measurements on hydrogenases, that these superclusters may also occur in proteins. In 1987 he obtained EPR evidence that an even larger cluster comprising 8Fe atoms was present in nitrogenase. This has recently been confirmed by X-ray crystallography of the 'P' clusters in this enzyme. In 1988 Hagen and his graduate student A.J. Pierik detected the same g-tensor, that was previously found in Coucouvanis prismane models, in a 6Fe containing protein. Some of the unusual EPR properties of this protein (notably very high spin states up to S=9/2) have been found by Hagen and his group in other complex redox enzymes: sulphite reductase, nitrogenase, CO dehydrogenase, suggesting that superclusters are quite common in nature. This area of research is now rapidly expanding and has initiated many successful collaborations particularly in the European Bio-inorganic Chemistry community.

At present Hagen is leading an active research group studying iron-containing proteins involved in sulphate-dependent respiration. He is also consulted by many research groups seeking help with the theory and practice of magnetic resonance spectroscopy of metalloproteins, with the theory and practice of protein electrochemistry and with the biochemistry of metal-containing biomolecules. On-going collaborations include direct electrochemistry of the Rieske protein, EPR and ESEEM of Ni(I) in factor F-430, redox titrations of the alternative (iron only) nitrogenase, electrochemical and EPR studies of slowly hydrolysing ruthenium anti-tumour drugs.

Hagen is the secretary of the Dutch Electrochemical Discussion Group and a member of the Dutch Bioenergetics working party. Since 1986, in addition to his University teaching commitments, he has been a lecturer at the annual FEBS advanced course on Inorganic and Physical Biochemistry held in Louvain.

Roger N.F. Thorneley

Secretary to the EUROBIC Steering committee

(Based on biographical details of Fred Hagen's career provided by his colleagues in The Netherlands)

NMR RELAXOMETRIC STUDIES OF HEMOPROTEINS: FROM THE CHARACTERIZATION OF THE METALLIC PARAMAGNETIC SITE TO CLINICAL APPLICATIONS

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The longitudinal relaxation rates (R_{1p}) of water protons of solutions containing ferric hemoproteins often showed a strong dependence upon temperature and pH. The increase of R_{1p} as the temperature increases was associated to the shortening of the exchange lifetime of the coordinated water molecule. Interesting differences in the exchange process have been observed in met-myoglobins of different origin, which has been ascribed to differences in the water accessibility to heme cavity. Furthermore the lack of any $^1H/^2H$ isotope effect on the exchange rate suggested that the displacement of the coordinated water molecule from Fe(III) is not the rate determining step in the overall exchange process.

The enhancement of water proton relaxation rate shown by met-Hb was exploited to suggest a new method for the quantitative *in vitro* assay of this species. Actually the dependence of R_{1p} by the exchange lifetime requires a careful control of the experimental temperature for an accurate determination of met-Hb percentage in human erythrocytes. This observation prompted us to look for an improved version of the relaxometric *in vitro* determination of met-Hb.

We suggest to transform met-Hb into the corresponding fluoromet-Hb (by adding an excess of NaF), which shows both an higher relaxivity and a negligeable dependence from the experimental temperature. The validity of the proposed method has been tested by the determination of the met-Hb percentage in several blood samples which has been checked by the clinically accepted spectrophotometric assay.

A Proton Relaxation Enhancement Study of the Non-Covalent Binding of Gd(III) Chelates to Human Serum Albumin.

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Gd-DTPA and Gd-DOTA complexes are the prototypes for hydrophylic contrast agents that do not interact with serum components and which are rapidly excreted through kidneys without significant uptake into body tissues. The introduction of aromatic moieties into the ligand structures allows these complexes to bind non-covalently to serum albumin, such interactions result in a markedly enhanced relaxivities (1,2). The extent of the enhancement depends upon a vast array of chemical and physical parameters, among which the number of aromatic residues is of primary importance.

In this regard, complexes containing benzyloxymethyl groups on a DOTA ligand skeleton (3) are good models to investigate binding with HSA. The strength of the interaction between these complexes and HSA (Sigma A9511) was determined from the K_d value for the equilibrium GdL-HSA $\leftrightarrow$ GdL + HSA .This was calculated in phosphate buffer 50 mM at pH 7.4 and 25°C, by measuring the Proton Relaxation Enhancement of aqueous solutions of the complexes and HSA over a wide range of concentration ratios. The $1/T_1$ NMRD profile of these complexes (0.1 mM) in presence of HSA (1.7 mM) reflected the occurence of such a strong interaction. As expected, when the lengthening of the molecular reorientational time (τ_r) allows the frequency dependence of the electronic relaxation time to be expressed, the profile of the bound form shows a relaxivity peak centered 20 MHz. However the relaxivity value measured at 25°C at the observation frequency of 20 MHz is remarkably lower than that expected on the basis of long τ_r (10^{-8} s) of HSA macromolecule. The behaviour of R_{1obs} versus temperature clearly indicates a role of the exchange lifetime τ_m in the observed relaxivity. As both τ_r and τ_m increase with decreasing temperature, the observed behaviour for these adducts with HSA can be accounted for by the absence of the fast exchange condition ($T_{1M} \ll \tau_m$).

References

1. Jenkins, B.J, Armstrong, E., Lauffer, R.B., *Magn. Res. Med.*, 17, 164, 1991.

2. Geraldes, C.F.G.C., Sherry, A.D., Lazar, I., Miseta, A., Bogner, P., Berenil, E., Sumegi, B., Kiefer, G.E., McMillen, K., Maton, F., Muller, R.N., *Magn. Res. Med.*, 3, 41 (1993).

3. Aime, S., Botta, M., Ermondi, G., Fedeli, F., Uggeri, F., *Inorg. Chem.*, 31, 6 (1992).

Chemical Characterization of Human Neuromelanin

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One of the possible causes responsible for the Parkinson's Disease is the occurrence of oxygen free radicals. Peroxides and superoxides arising from molecular oxygen are not particularly toxic, but they can be activated to OH· radicals in the presence of the redox iron pair through the Haber - Weiss cycle. These radicals act as starters of the lipid peroxidation with the subsequent cellular damage. Thus, every molecular system effective in changing both iron concentration and oxidation state could be effective in modulating the extent of lipid peroxidation. Melanin present as the black pigment in Substantia Nigra Pars Compacta can act in this way. Two hypothesis on the role of neuromelanin have been forwarded: neurotoxic and neuroprotective. Since these hypothesis are principally based on studies carried out on synthetic model melanins, the primary aim of our work was tthen to get more insight into the chemical characterization of the human mesencephalic neuromelanin.

The most remarkable finding in the carbon NMR solid-state spectrum of a human neuromelanin is the presence of strong absorptions in the "lipid" and "carbohydrate" regions. Moreover, the iron content in this melanin is 100 times higher than in other natural melanins (e.g. from Sepia ink), whereas the copper concentration is quite similar. On comparing our results with previously reported Substantia Nigra Iron concentrations, we might assume that all iron present in Substantia Nigra is bound to neuromelanin.

Furthermore, we found that most of iron is so tightly bound to the melanin-glycidic-lipidic matrix that it cannot be removed by DTPA. This assembly, constituting neuromelanin, is quite resistant to the attack by H_2O_2, in contrast with the behaviour shown by melanins of different origin.

Our results suggest therefore that, in non-pathological conditions, iron can be stored by this system, which is sufficiently stable to resist to the attack of OH· and oxidants. Stored iron is no longer available for catalyzing the Haber-Weiss cycle. Under pathological conditions, this molecular assembly could be damaged, releasing stored iron and starting the neuronal degeneration.

Spectroscopic characterisation of three average-valence dicopper cryptates

A H R Al-Obaidi[a], J A Farrar[b], J J McGarvey[a], V McKee[a], J Nelson[a,c] and A J Thomson[b]

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Accumulating evidence[1] that the electron-transfer Cu_A site in nitrous oxide reductase and cytochrome oxidase contains an average-valence dicopper assembly has stimulated interest in mixed-valence dicopper models.

Recently, three mixed-valence dicopper cryptates have been structurally characterised [2]; their 4K esr spectra (adjacent poster) demonstrates that they are delocalized Class III systems: thus,more acurately average-valence dicopper(1.5). Each copper cation occupies a trigonal bipyramidal coordination site, separated by 2.45-2.41 Å from its neighbour, this separation representing a one-electron bond. (Fig 1[a-c])
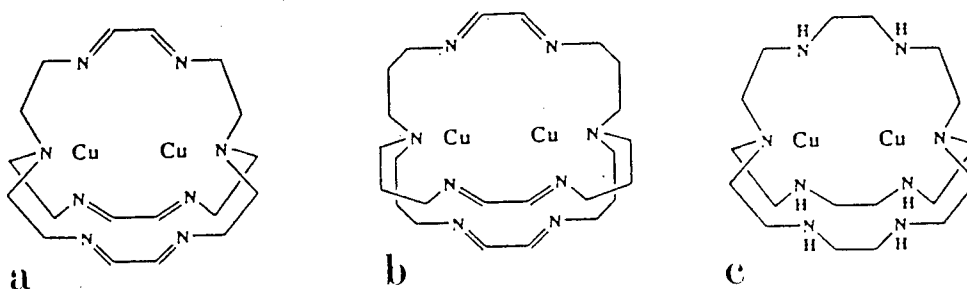
The interesting and unusual electronic spectral properties(absorption and MCD spectra) which characterise this unusual bonding situation are here reported, together with a study of the resonance Raman spectra excited by irradiation into their near ir and UV absorptions.

References
(1)   G C Steffens, R Biewald and G Buse, *Eur J Biochem* , 1982, **164** 295; P M H Kroneck, W E Antholine, D W H Kastrau, G Buse, G C Steffens and W G Zumft, *FEBS Lett.*, 1990 **268** 274; J A Farrar, A J Thompson, M R Cheesman, D M Dooley and W G Zumft *FEBS Lett.*, 1991 **294** 11.
(2)   C Harding, V McKee and J Nelson, *J Am Chem Soc.*, 1991 **113** 9864; M E Barr, P H Smith, W E Antholine, B Spencer, *J Chem Soc., Chem Commun.*, 1993, 1649 ; J A Farrar, V McKee, A H R al-Obaidi, J J McGarvey, J Nelson and A J Thompson, *Inorg. Chem.*, in press.

POTENTIOMETRIC AND SPECTROSCOPIC STUDIES ON OXOVANADIUM(IV) PHOSPHONATE COMPLEXES

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The chemistry of vanadium within biological systems highlights the central role played by phosphate ligands in complexing and thus stabilizing oxovanadium(IV) inside the cell, after the metal ion has passed through cellular membranes as vanadate(V) and it has been reduced in order to prevent the toxic effects of the higher oxidation state.

Continuing our research on the complexing properties of phosphate ligands towards the oxovanadium(IV) ion, we have turned our attention to phosphonate ligands and compared them with the analogous carboxylates.

The combined application of potentiometric and spectroscopic (EPR and electronic absorption) techniques shows that, differently from monophosphate, which binds the metal ion forming four-membered chelate rings, methylphosphonate acts only as a monodentate ligand so that hydrolysis sets in at very low pH. The introduction of an auxiliary coordinating function, -OH or -COOH, allows for chelation and formation of both mono and bis complexes, thus protecting the metal ion from hydrolysis, at least over the acid pH range. With phosphonoformic acid even a dimeric species is observed. Other ligands have been considered: phosphonoacetic acid, which is the higher homologous of phosphonoformic acid, methylenebis(phosphonic) acid and 1-hydroxyethylidene-bis(phosphonic) acid. Noteworthy, there is spectroscopic evidence of methylenebis(phosphonic) acid binding the oxovanadium ion to yield a trinuclear species similar to the already well-characterized analogous formed by diphosphate.

Vanadium haloperoxidases from *Laminaria* of the portuguese coast

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Vanadium haloperoxidase enzymes were reported for several algae(1), as well as for terrestrial fungi (2) and one lichen (3). These enzymes are of potential technological interest(4).

In the Portuguese coast there exists a wide variety of algae. Several species of *Laminaria* contain this type of enzymes (for instance *Laminaria saccharina*(5)). We present here a comparative study for two species of the Laminariaceae.

Haloperoxidases from the brown alga *Saccorhiza polyschides* Batters and *Laminaria hyperborea* Foslie were isolated by a liquid-liquid extraction procedure(6) using an aqueous-two-phase system containing potassium carbonate and polyethyleneglycol 1500.

Three isoenzymes were isolated, from the alga *Saccorhiza polyschides* showing different vanadium dependent iodoperoxidase activity. No bromo or cloroperoxidase activity was observed for these isoenzymes.

Preliminary studies showed that *Laminaria hyperborea* also presents a specific iodoperoxidase non-hemic activitiy, higher than that obtained for the isoenzymes isolated from the alga *Saccorhiza polyschides*.

Comparative characterization based on specific inactivation/reactivation studies with vanadium, determination of molecular mass using poliacrylamide gel electrophoresis and H.P.L.C. chromatografic methods, determination of catalytic kinetics parameters at various pH and spectroscopic techniques will be presented .

(1) Vilter, H.; Glombitza, K.-W. ; Grawe, A. *Botanica Marina* (1983), **26**, 331-340.
(2) van Schijndel., J.W.P.M., Vollenbroek, E.G.M., Wever, R. *Bioch. Biophy. Acta* (1993), **1161**, 249
(3) Plat H., Krenn, B.E., Wever, R. Biochem. J. (1987), **248**, 277-279.
(4) Chasteen, N.D. "Vanadium in Biological systems", Kluwer Academic Publisher, Dortrecht (1990).
(5) de Boer, E., Tromp. M.G.M. ,Plat, H., Krenn, B.E., Wever, R. *Bioch. Biophy. Acta* (1986), **872**, 104-115.
(6) Jordan, P.; Vilter, H. *Bioch. Biophy. Acta* (1991) **1073**, 98-106.

Complementary EPR, Mössbauer and Susceptibility Investigations
on Model Compounds of Heme Peroxidases

J. Anthony, E. Bill, M. Gerdan, M. Grodzicki, M. Kröckel, M. Müther, A.X. Trautwein[a], A. Gold[b],
D. Mandon, Th. Wolter, R. Weiss[c]

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Compound I and compound II are intermediates in the reaction cycle of heme peroxidases. They represent high-valent iron complexes which both contain the ferryl $(Fe(IV)=O)^{2+}$ unit. In compound I, the porphyrin is oxidized, comprising an oxoferryl porphyrin π-cation radical. Compound I and its synthetic analogues are spin-coupled systems with exchange interaction between ferryl iron $(S=1)$ and porphyrin radical $(S'=1/2)$. Despite general similarities in physicochemical properties the strength of exchange in different complexes varies considerably, generating spin coupling schemes, which range from antiferromagnetic to ferromagnetic coupling. The coupling constant J appears to represent a key parameter of the electronic structures. Reliable investigations of zero-field interaction of ferryl iron and exchange coupling of the compound-I systems require the application of complementary spectroscopic techniques like Mössbauer and EPR spectroscopy, magnetic susceptometry in conjunction with spin-Hamiltonian simulations, as well as molecular orbital calculations.

We were studying synthetic analogues of compound I and II. In this contribution we will present spectroscopic and theoretical results of several systems, while the chemical approaches of this work are presented elsewhere in this volume [1,2]. The examples are selected to cover a range of systematic variations in exchange interaction. We will demonstrate the experimental analyses and elucidate ambiguities, arising from the competition of zero-field and exchange interaction. Correlations of spin-Hamiltonian parameters and structural specificities, as well as exchange pathways in peroxidase compound I analogues will be delineated.

1. Ayougou K., DeVos R., Wolter T., Mandon D., Weiss R., Muether M., Bill E., Butzlaff Ch., Trautwein A.X., Austin R.N., Jayaraj K. and Gold A., 1994, this volume
2. Weiss R., 1994, this volume

Synthesis, Characterization, Absorption Spectra, and Luminescence Properties of Platinum(II) terpyridine Cationic Complexes.

ARENA, G.; CALOGERO, G.; CAMPAGNA, S.; MONSU' SCOLARO, L. ; RICEVUTO, V.; ROMEO, R.

Contribution from Dipartimento di Chimica Inorganica , Analitica e Struttura Molecolare, University of Messina, Messina, ITALY.

A series of cationic complexes of platinum(II) containing terpyridine ligands of the type [Pt(R-terpy)X]Cl (R = H, Ph; X = Cl, Me, Ph) were synthesized and fully characterized by ^{1}H, ^{13}C and ^{195}Pt NMR spectroscopy. The terpyridine behaves as a tridentate ligand and no evidence of fluxionality has been found. ^{1}H NMR data are strongly indicative of a dimerization process in aquoeus or alcoholic solution, as observed previously for other square planar compounds containing the same ligand [1] and in organometallic species with R = H and X = Me [2]. When X is a phenyl group the formation of the dimer is hindered.
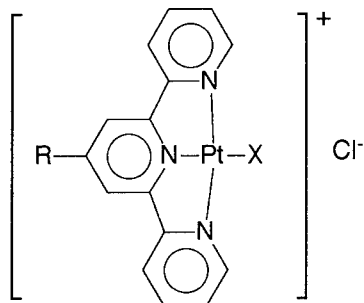
The absorption spectra are dominated by intense bands in the UV region (ε in the range 10^4 - 10^5 M^{-1}cm^{-1}) attributed to terpy-centered (LC) transitions, and by moderately intense bands in the visible (ε in the range 10^3 - 10^4 M^{-1} cm^{-1}) assigned to metal-to-ligand charge transfer (MLCT) transitions.

All the compounds are strongly luminescent at 77K in MeOH/EtOH 4:1 (v/v) rigid matrices from LC and/or MLCT excited states (emission maxima in the range 500 - 600 nm, lifetimes on the microsecond time scale). Except for X = phenyl, the complexes exhibit a second luminescence band at lower energies, that can be attributed to a Pt-Pt σ^* to polypyridine ligand CT level.

Most of the compounds are moderately luminescent in deoxygenated solutions even at room temperature. The emission bands are in all cases red-shifted with respect to the low temperature emission bands, confirming the charge transfer nature of the processes. The presence of the lower energy band supports the formation of dimeric species.

The structural and photophysical properties of these complexes are being investigated for their use as probes for nucleic acids.

(1) Jennette, K.W.; Gill, J.T.; Sadownick, J.A.; Lippard, S.J. *J. Am. Chem. Soc.* **1976**, *98*, 6159-6168. Lippard, S.J. *Acc. Chem. Res.* **1978**, *11*, 211-217.

(2) manuscript in preparation

EPR REDOX TITRATION OF HYDROGENASE FROM THE HYPERTHERMOPHILE *PYROCOCCUS FURIOSUS*

A.F. Arendsen, P.Th.M. Veenhuizen and W.R. Hagen
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The hydrogenase of the extreme thermophile *Pyrococcus furiosus* has been characterized by Bryant and Adams [1]. EPR spectroscopy exhibited a near-axial signal in the reduced state at 70 K with resonances at g= 2.03; 1.93 and 1.92. This signal added up to only one spin/mole. Upon lowering the temperature to 10 K a complex spectrum appeared with very rapid spin relaxation properties, which accounted for another spin/mole. Adams reports the monitoring of a 4Fe and a 2Fe cluster in a redox titration, the midpoint potentials being -410 mV and -210 mV, respectively [2].
We have re-investigated the EPR properties of the *P. furiosus* enzyme. In the oxidized state the enzyme is EPR silent. In a mediated, reductive titration a novel rhombic signal appears with (**A**) E_m = -106 mV. Further reduction causes the rhombic signal to disappear with E_m = -329 mV and concomitantly the previously reported [1] sum spectrum appears of a near-axial (**B**) plus a complex signal (**C**). The titration is interpreted as two subsequent one-electron reductions
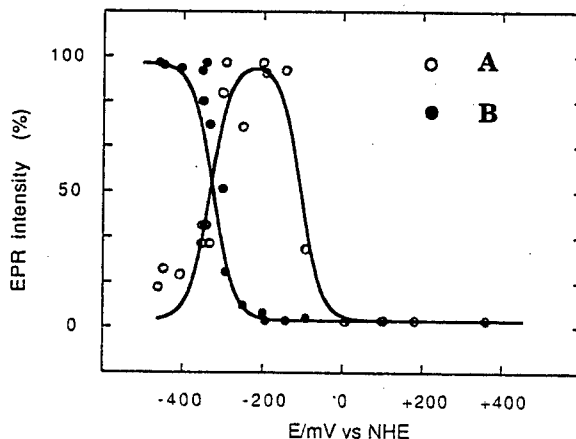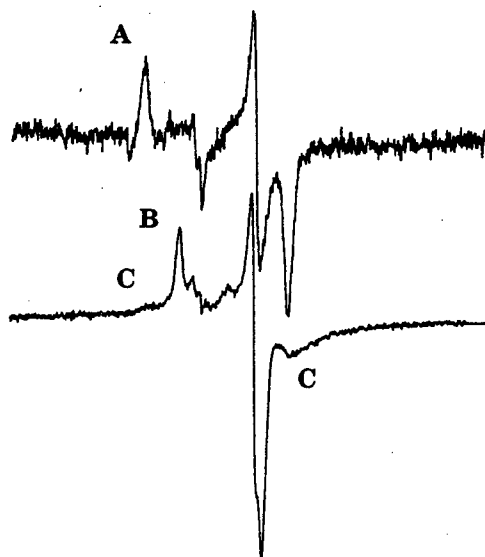
1. F.O. Bryant and M.W.W. Adams, *J. Biol. Chem.*, 264, 5070-5079 (1989).
2. M.W.W. Adams, *Advances in Inorganic Chemistry*, 38, 345-250 (1992).

POSSIBILITIES FOR VOLTAMMETRIC METHODS IN MECHANISTIC STUDIES OF COMPLEX ELECTRON TRANSPORT ENZYMES

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Multi-centred redox enzymes such as respiratory complexes present a formidable challenge with regard to elucidating how catalytic action is executed, regulated, and harmonised. By linking the enzyme electrostatically to a modified electrode surface in such a manner that interfacial electron exchange is facile, the catalytic electron transport characteristics of these systems becomes determinable in the same way as one examines the performance of an electronic circuit. We review observations that have been made with succinate dehydrogenase (beef heart mitochondria) and fumarate reductase (*E. coli*) and outline some possibilities for detailed mechanistic investigations of other important systems.

1. Diode-like Behaviour of a Mitochondrial Electron-transport Enzyme.
A. Sucheta, B. A. C. Ackrell, B. Cochran & F. A. Armstrong. *Nature 356*, 361 (1992).

2. Reversible Electrochemistry of Fumarate Reductase Immobilized on an Electrode Surface. Direct Voltammetric Observations of Redox Centers and their Participation in Rapid Catalytic Electron Transport. A. Sucheta, R. Cammack, J. Weiner & F. A. Armstrong. *Biochemistry 32*, 5455 (1993).

3. Classification of Fumarate Reductases and Succinate Dehydrogenases based upon their Contrasting Behaviour in the Reduced Benzylviologen/Fumarate Assay. B. A. C. Ackrell, F. A. Armstrong, B. Cochran, A. Sucheta & T. Yu. *FEBS Lett. 326*, 92 (1993).

Interactions of Aluminium(III) with Biologically Important Phosphates

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Soluble aluminium species have been implicated in a number of toxic biological processes in plants and humans. More recently aluminium(III) has been found to influence the activity of a range of phosphatase enzymes. The mode of aluminium(III) action is uncertain, but a recurring theme is its association with phosphate groups. It is very likely that aluminium(III) binds to basic phosphate groups that occur in phosphorylated proteins and nucleoside phosphates. Since the average total phosphate concentration of body fluids is 4.5 mM, organic and inorganic phosphates may be significant aluminium(III) carriers in humans. Therefore, besides a series of organic monophosphates, the study was extended to the inorganic phosphates; monophosphate (MP), diphosphate (DP) and triphosphate (TP).

pH−Potentiometry was used to determine the stoichiometries and stabilities of the complexes formed in various Al(III)−biophosphate systems and ^{1}H and ^{31}P NMR measurements to support the solution speciation results. The quantitative characterization of the Al(III)−MP interaction is especially difficult because of AlPO$_4$ precipitation. To overcome this problem, the linear relationship between the pK$_a$ of various nucleoside monophosphates (AMP, CMP, GMP, TMP, UMP) and other organic phosphates and the log K$_{AlA}$ values of their soluble Al(III) complexes of the same bonding mode was used to assess the stability of the AlHPO$_4$$^+$ species. Satisfying our expectations, a straight line was obtained with a slope of ~0.7. The points for AMP and CMP, however, did not fit the line, the stabilities of their corresponding Al(III) complexes are greater than expected. An explanation of the enhanced stability of these complexes might lie in the presence of an aromatic nitrogen donor in AMP and CMP, which could somehow increase the strength of their interaction with Al(III).

DP and TP, as multidentate biophosphates bind Al(III) much more strongly, than monophosphates, and besides normal mono and bis chelates, they form various protonated and deprotonated/hydroxo complexes.

EPR Studies of [Fe(II)Fe(III)] and [Fe(II)Fe(II)] Clusters Formed in the R2 Subunits of Ribonucleotide Reductase
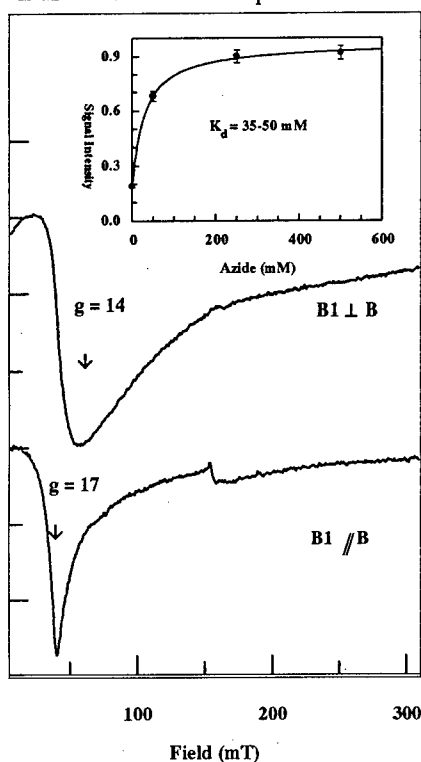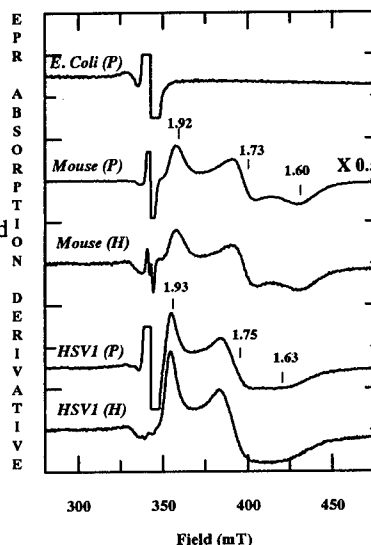
Atta, M.,[*] Andersson, K.K.[*], Debaecker, N.[#], Latour, J-M.,[#] Thelander, L.[§], Gräslund, A.[*].

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Ribonucleotide reductase (RNR) catalyses the reduction of all four ribonucleotides to their corresponding deoxyribonucleotides, an essential reaction in all living cells. The smaller R2 subunit contains a stable tyrosyl free radical and a binuclear iron centre within each polypeptide chain. We report EPR spectra (right Figure) at 4 K of the mixed valent Fe(II)Fe(III) state of R2 proteins from mouse (g = 1.92, 1.73, 1.60) and herpes simplex viruses type 1 (HSV1) (g = 1.93, 1.75, 1.63). Observation of these EPR signals with g_{ave} below g = 2.00 is a solid proof for the presence of dinuclear iron clusters with (hydr)oxybridges, which are known to exist in the *Escherichia coli* R2 protein. Two mild reduction methods (**P** and **H**) are used to obtain mixed valent states. **P** is after incubation with phenazine methosulfate and dithionite, and **H** is

Field (mT)

Field (mT)

after incubation with 5 mM hydrazine, both at pH 7.5. We estimate the coupling constant J ($H = J*S_1*S_2$) to be J = -15 cm^{-1} for the mouse protein, and J = -11 cm^{-1} for the HSV1 protein. This is an indication that the presumed oxybridge in the diferric state is converted into a hydroxy-bridge in the mixed valent state. Thus there are large differences in redox behaviour between the *E. coli* iron cluster in its R2 protein and the two clusters in mouse and HSV1 R2 proteins [1]. We have observed the diferrous form of mouse RNR R2 protein by integer spin EPR. Typical spectra are shown in the left Figure. In absence of glycerol the S = 4 EPR signal increased with addition of azide (see inset). The presence of glycerol also induces a strong EPR signal. Low temperature magnetic susceptibility measurements show weak coupling of the diferrous state.

[1] Atta *et al.* (1994) *J. Am. Chem. Soc*, (submitted)

SPIN COUPLING IN COMPOUNDS I OF THE PEROXIDASES: A CHEMICAL APPROACH

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In the compounds of type I of peroxydo-dependent hemoproteïns, the overall oxidation state at the active site is two equivalents above ferric: a ferryl-oxo ion of spin $S_1 = 1$ is present, together with an organic radical having a spin $S_2 = 1/2$; this radical may be located on the prosthetic group or on an amino-acid residue. A phenomenological model with the coupling interaction $S_1.J.S_2$ between these two spins is most often used to describe the magnetic properties of these species. J may vary in direction and magnitude, and is actually the resultant of ferromagnetic (J_F) and antiferromagnetic (J_{AF}) contributions.

The highly oxidized intermediates in biological systems display various magnetic behaviours, in spite of the presence of the same prosthetic group (protoporphyrin IX): a significant antiparallel coupling is observed in chloroperoxydase[1] and catalase displays a weak parallel interaction[2]; an extremely weak interaction takes place in horseradishperoxidase[3]; no interaction at all is detected in cytochrome c peroxidase whereas the radical is located on a cluster of several amino-acid residues[4]. The reasons for such different magnetic behaviours are still poorly understood.

We have been studying synthetic analogues of compounds of type I using a set of different spectroscopies. We have already shown that with simple tetraarylporphyrins a parallel interaction of moderate magnitude takes place[5]. We have also worked with porphyrins having functional groups in the vicinity of the ferryl ion. We know in that case, that these functional groups are partially oxidized (they may share the oxidation with the macrocycle) and interact through space with the ferryl ion in a very weak fashion.

We are now examining several parameters such as:
the distortion of the porphyrin
the symmetry around the coordination site
the nature of the porphyrin (tetraarylporphyrin vs. meso-free porphyrin)
the axial coordination
the presence of protic and oxidizable functional groups close to the coordination cavity

and try to study their incidence on the magnetic exchange in oxo-ferryl radical species. We shall present exemples from our most recent results.

1   Rutter, R.; Hager, L. P.; Dhonau, H.; Hendrich, M.; Valentine, M.; Debrunner, P., *Biochemistry*, **1984**, 23, 6809.
2   Benecky, M. J.; Frew, J. E.; Scowen, N.; Jones, P.; Hoffman, B. M., *Biochemistry*, **1993**, 32, 11929.
3   Schulz, C. E.; Rutter, R.; Sage, J. T.; Debrunner, P. G.; Hager, L. P., *Biochemistry*, **1984**, 23, 4743.
4   Sivaraja, M.; Goodin, D. B.; Smith, M.; Hoffman, B. M., *Science*, **1989**, 245, 738.
5   Mandon, D., Weiss, R.; Jayaraj, K.; Gold, A.; Terner, J.; Bill, E.; Trautwein, A. X., *Inorg. Chem.*, **1992**, 31, 4404.

COPPER UPTAKE IN YEAST INDUCED BY VARIOUS STRESS CONDITIONS

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Copper is an essential element and has multiple functions in redox enzymes. On the other hand, excess copper is toxic to eucaryotic as well as procaryotic cells. Alterations of copper concentrations in tissues or fluid have been reported for many diseases. Further, copper metabolism proved to be sensitive to various chemicals causing oxidative stress in both yeast and mammalian cell cultures.

The aim of this work was to gain insight into the mechanisms and consequences involved in the observed copper accumulation induced by stress, using the yeast *Saccharomyces cerevisiae* as an experimental system.

A massive increase of copper levels was brought about by treating the cells with

- elevated copper concentrations in the medium (300 µM)
- the redox cycling agent menadione (200 µM)
- iron deficiency
- heat shock.

Copper uptake from copper incubation compared with the other treatments differed in several biochemical and physiological features:

- In media with high copper concentrations the uptake occurred immediately whereby the accumulation induced by menadione showed a delay of about one hour
- Strongly copper binding ligands as d-penicillamine or triethylenetetramine were able to suppress the extended accumulation produced by menadione but not by copper
- Cycloheximide, a protein synthesis blocking agent, did not prevent copper uptake under elevated copper concentration. However, it inhibited the copper uptake usually observed by menadione or heat shock exposition
- Additional copper in cells incubated with 250 µM copper was mainly localized in vacuoles. In the menadione system it stayed to a great extent in the cell wall.

The results suggest two different mechanisms of copper uptake. With elevated medium copper concentrations, uptake seems to follow the normal pathway. The accumulation induced by menadione, however, is dependent on the synthesis of a protein which indeed binds a high amount of copper, but with a low affinity.

The Design of Sterically Restrictive Metal Complexes and Their Binding to DNA Constituents.

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The nature of the binding of a metal complex to a nucleic acid
may be significantly influenced by steric factors. In some cases
intramolecular steric effects may even be determinative of the
molecular conformation of the adduct [1]. The manipulation of the
steric parameters on the "carrier ligands" of metal complexes
which bind to DNA may hold promise for the rational design of
reagents which produce predictable distortions in nucleic acids,
and may provide a means for the control of various functions
related to nucleic acid topology [2]. Such reagents may also find
uses as tags or probes [3].

One possible way of manipulating the geometry of cis-coordinated
nucleobases is to use a carrier ligand which specifically blocks
off one side of a square-plane. This arrangement has the
potential to enforce a so-called "head-to-head" (HH) orientation
of coordinated nucleobases. Subsequent structural modifications
of the carrier ligand could result in a wide range of
conformations which could be reflected in the tertiary structure
of a coordinated nucleic acid.

To explore the above, several classes of bidentate carrier
ligands complexed to metal ions are under investigation, one of
which, (1,2-Bis-(6-methylpyridine-2-yl)ethane-N,N')-
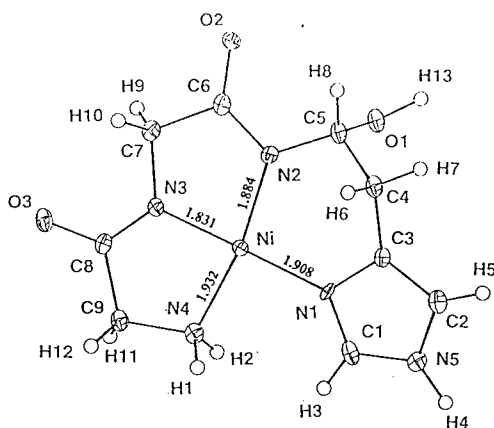palladium(II) is presented here [4].

1. J.D. Orbell, L.G. Marzilli, and T.J. Kistenmacher, *J. Am.
   Chem. Soc, 103* (1981) 5126.
2. P.M. Pil and S.J. Lippard, *Science, 256* (1992) 234.
3. A.M. Pyle and J.K. Barton, in S.J. Lippard (ed.), *Progress in
   Inorganic Chemistry*, Vol. 38, Wiley, New York, 1990, p.413.
4. A.T. Baker, J.K. Crass, G.B. Kok, J.D. Orbell and E. Yuriev,
   Inorg.Chem.Acta, 214 (1993) 169.

Peptide Modification by Ni(III): O₂-Induced Decarboxylation and Dehydration of [Ni(II)(Gly-Gly-L-His)]

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Ni(II) and Cu(II) peptide complexes can catalyze the oxidation and cleavage of DNA [1]. We have found that O_2 can induce two types of peptide modification of [Ni(II)(GGH-H$_{-2}$)] via

Ni(III) intermediate. The first gives [Ni(II)(Gly-Gly-α-hydroxy-D,L-histamine)].3H$_2$O, as confirmed by X-ray crystallography [2]. In the presence of excess O_2, the product is [Ni(II)(Gly-Gly-α,β-didehydrohistamine-H$_{-2}$)] which has been characterized by NMR spectroscopy.

1. D.F. Shullenberger, P.D. Eason, and E.C. Long, *J. Am. Chem. Soc.* 1993, **115**, 11038.

2. W. Bal, M.I. Djuran, D. W. Margerum, E. T. Gray, Jr., M. A. Mazid, R. T. Tom, E. Nieboer, and P. J. Sadler, submitted for publication

EFFECT OF NEGATIVELY CHARGED MODEL MEMBRANES ON METAL- PEPTIDE INTERACTIONS.

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Studies on model systems and structure-relationships indicate a regulatory role of negatively charged biological membranes in the binding of peptide hormones to receptors [1]. In order to investigate a membrane's potential for a similar role in peptide-metal ion interactions, protonation and Cu(II) binding to several oligopeptides in the presence of micellar solutions of SDS (sodidum dodecyl sulfate) was studied by potentiometry and NMR, EPR and CD spectroscopies. Following results were obtained [2]:

Peptides containing small or hydrophilic side chains (GGGG, AAAA, ADSG) showed only a slight elevation of protonation constants (0.2-0.3 log units) in the presence of 0.1 M SDS, compared to 0.1 M NaCl. Their Cu(II) complexes showed a small but uniform loss of stability, reflected by the decrease of stability constants by 0.2-1.0 log units. These effects indicate that neither these peptides nor their complexes interact with SDS micelles, and numerical differences are due to a non-specific ionic attraction of both protons and cupric ions by SDS. Protonation constants of peptides containing both hydrophobic residues and positively charged Arg side chains (peptide hormones LHRH, AVP, SP) were elevated by up to 2 log units, and their complexation pattern was markedly altered. AVP has high affinity to the micelle, whereas its cupric complexes are unable to bind to it. In the result, SDS micelles prevent Cu(II) binding to AVP below pH 8, an increase of ca. 3 pH units compared to NaCl solutions. On the contrary, ability of LHRH to bind Cu(II) is hardly affected by SDS, despite the elevation of protonation constants. The dominant complex species over the pH range 6.5-11 is similar to that of NaCl solutions (a 3-nitrogen co-ordination at N-terminal Glp and His), the peptide molecule is, however, held in a specific conformation at the micelle, locating the Cu(II) ion near its surface.

The results presented above indicate that a negatively charged membrane can be a specific selection factor in the metal ion - peptide hormone interactions.

1. R.Schwyzer, *Biopolymers*, 1991, **31**, 785.
2. W.Bal, H.Kozlowski, M.Lisowski, L.D.Pettit, R.Robbins and A.Safavi, *J.Inorg.Biochem.* , in press.
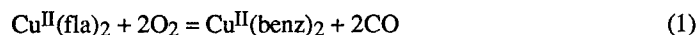
KINETIC STUDIES IN MODEL QUERCETINASE REACTIONS USING COPPER(II) FLAVONOLATE COMPLEXES

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The copper(I) and copper(II) flavonolate complexes catalyze the oxygenation of flavonol in DMF resulting in the selective formation of depside (phenolic carboxylic acid ester) and carbon monoxide [1,2]. These systems provide the first copper-containing models for quercetinase, a Cu^{II}-containing dioxygenase.

In order to disclose the ring-cleavage reaction in flavonol we reacted $[Cu^{II}(fla)_2]$ (flaH =f lavonol) with dioxygen in acetonitrile resulting in bis(O-benzoylsalicylato)copper(II), $[Cu^{II}(benz)_2]$ (benzH = O-benzoylsalicylic acid) and carbon monoxide quantitatively. The complex was characterized by spectroscopic methods and X-ray analysis (eq. 1).

$$Cu^{II}(fla)_2 + 2O_2 = Cu^{II}(benz)_2 + 2CO \qquad (1)$$

The kinetic data of the oxygenation of $[Cu^{II}(X-fla)_2]$ (X= H, 4'-OMe, 4'-OH, 4'-Cl, 4'-Me, 4'-NO$_2$) in DMF show first order dependence with respect to the complex and dioxygen suggesting that the rate-determining step is the addition of dioxygen to the coordinated flavonol (eq. 2). The reaction fits a Hammett linear free energy relationship, and an increase of the electron density on copper(II) makes the oxygenation easier.

$$\text{reaction rate} = k_2\,[Cu^{II}(X-fla)_2]\,[O_2] \qquad (2)$$

Results of the kinetic measurements of the oxygenation of flavonol using the compound $[Cu^{II}(fla)_2]$ suggested that the rate-determining step is preceeded by the reaction of flavonol with the catalyst to give an intermediate complex. The coordinated flavonolate reacts then with dioxygen, and probably an endoperoxide is formed, which breaks down in fast consecutive reactions to a O-benzoylsalicylatocopper complex.

1. G. Speier, V. Fülöp and L. Párkányi, *J. Chem. Soc., Chem. Commun.*, 512 (1990)
2. É. Balogh-Hergovich, G. Speier and Gy. Argay, *J. Chem. Soc., Chem. Commun.*, 551 (1991)

STRUCTURAL CHARACTERIZATION IN SOLUTION OF MUTANTS OF THE MONOMERIC FORM OF SOD THROUGH NMR SPECTROSCOPY

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The understanding of the relation between the structural properties of a biological molecule and its function is one of the fondamental steps in its complete characterization.

In the case of proteins formed by two or more identical subunits a further question arises about the factors determining the subunit-subunit interaction and the effects of this interaction on the structure of the protein.

Recently, the monomeric form of Coppper,Zinc superoxide dismutase (SOD) has been obtained by modifying residues at the dimer interface. Additional mutations at the active site have been also obtained.

SOD, which catalyzes the dismutation of superoxide to molecular oxygen and hydrogen peroxide, is a dimeric enzyme in which each subunit contains, in the active site, a copper and a zinc ions. The active site channel, which connects the bulk solution with the site of the reaction, is characterized by the presence of several charged residues.

The half molecular weight (16,000 with respect to 32,000 for WT SOD) of the monomeric form of SOD allowed us to tackle the structural characterization of SOD in solution through NMR.

We will present here the ^{1}H and ^{15}N NMR assignment for the reduced form of the mutant at position 133 of the monomeric analog of SOD, which is constituted by 153 residues. The assignment has been obtained though 2D NOESY, TOSCY and COSY experiments as well as through 3D NOESY- ^{1}H ^{15}N HMQC and TOCSY -^{1}H ^{15}N HMQC experiments.

From the NMR characterization, structural information are obtained on the entire protein as well as on the active site and the channel to the reaction site.

Proximal and Distal Site Mutants of Cytochrome c Peroxidase

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Cytochrome c peroxidase (CcP) is a heme enzyme (MW=34,000) that catalyzes the oxidation of ferrocytochrome c by H_2O_2 through formation of an intermediate (compound I) that is oxidized by two equivalents above the resting enzyme. The resting enzyme possesses a five coordinate, high spin iron(III) heme center with a proximal His axial ligand.

Despite extensive structural characterization of this enzyme, several questions still require clarification to achieve a comprehensive understanding of the relationship between the structure of this protein and its function. These questions include identification of the factors that dictate the reduction potential of the heme iron, the origin of the pH-dependence of enzymatic activity, and the mechanism by which small aromatic substrates bind to the enzyme. We have studied some of these questions by investigating several active site variants of CcP.

For example, we have replaced Asp235, which forms a hydrogen bond with the proximal His residue and influences the Fe-His bond and reduction potential, with an Ala residue.[1] The Asp235Ala variant exhibits three spectroscopic species between pH 5 and 9: a high-spin form that dominates at low pH and two low-spin species that form successively at higher pH. Disruption of the hydrogen bond between the proximal histidine residue and Asp235 reduces the strength of the interaction between the His ligand and the iron, accounting for the increase in reduction potential for the Fe(III)/Fe(II) couple observed for this variant. Replacement of Trp51, a large residue present in the distal heme pocket where reaction with H_2O_2 occurs, with a small residue such as Ala creates a space for the binding of small aromatic molecules. From the analysis of the ^{1}H NMR spectra of this variant in the presence of styrene, catechol and guaiacol, we have found that such molecules bind close to the heme but in slightly different arrangements that correlate with the differing hydrophobicities of the three aromatic compounds. Moreover, although Trp51 does not directly interact with the heme iron or with ligands bound to the iron, its replacement strongly affects the pH-dependent properties of the active site. Finally, we have changed the positively charged Arg48 present in the distal heme pocket to a negatively charged Glu residue and found that this substitution strongly influences the pH-dependent coordination properties of heme iron. The implications of these studies for the contributions of each of the modified residues to the mechanism of CcP will be discussed.

1) Ferrer, J.C.; Turano, P.; Banci, L.; Bertini, I.; Morris, I.K.; Smith, K.M.; Smith, M.; Mauk, A.G. *Biochemistry*, **1994**, in press.

^{1}H and ^{15}N NMR assignment [1] and solution structure [2] of the reduced form of the iron-sulfur protein iso I (HiPIP I) from *E. halophila,* expressed in *E. coli.*

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Dieter H. W. Kastrau, Claudio Luchinat, Mario Piccioli, Roberta Pierattelli,
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The recombinant high potential iron sulfur protein (HiPIP) I from *Ectothiorhodospira halophila* expressed in *E. coli.* contains 73 aminoacids and a 4Fe4S cluster as prostetic group. In the reduced form, the $[Fe_4S_4]^{2+}$ cluster has no unpaired electrons in the ground state but the contribution of the excited states at room temperature yields a paramagnetism of about 0.8 μ_B per iron ion. The paramagnetic effects on nuclear relaxation are so strong as to yield T_1 values of few milliseconds and linewidths of hundreds Hz for the nuclei closest to the paramagnetic center.

Despite these features, through classical methods and experiments optimized for detecting connectivities among fast relaxing signals, 71 out of 73 residues were assigned, accounting for 88% of protons and for 84% of nitrogen signals.

From NOESY intensities a family of structures has been proposed and the refinement has been performed using the experience of the Florence laboratory in handling open shell metal ions. The final results are quite satisfactory.

[1] Ivano Bertini, Isabella Felli, Dieter H. W. Kastrau, Claudio Luchinat, Mario Piccioli and Maria Silvia Viezzoli: Sequence-specific assignment of the ^{1}H and ^{15}N Nuclear Magnetic Resonance spectra of the reduced recombinant high potential iron sulfur protein (HiPIP) I from *Ectothiorhodospira halophila,* European Journal of Biochemistry, in press

[2] Lucia Banci, Ivano Bertini, Lindsay D. Eltis, Isabella Felli, Dieter H. W. Kastrau, Claudio Luchinat, Mario Piccioli, Roberta Pierattelli and Michael K. Smith: The three dimensional structure in solution of the paramagnetic protein HiPIP I from *E. halophila* through Nuclear Magnetic Resonance, European Journal of Biochemistry, in press

The Interaction of Peroxidases with Substrates and Mediators studied by NMR Spectroscopy

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Heme proteins constitute a very wide class of macromolecules which perform a large variety of biological functions. Peroxidases are heme-containing enzymes which can oxidize several types of substrates through two-electron oxidation. The substrate can be constituted by simple aromatic molecules in the case of horseradish peroxidase (HRP), or by large polymeric molecules such as lignin in the case of lignin peroxidase (LiP) or manganese peroxidase (MnP), or by another protein such as cytochrome c in the case of cytochrome c peroxidase (CcP). In some systems the reaction occurs through a mediator, *i.e.* a small molecule which is oxidized by the enzyme and which, on its turn, is able to oxidize the real substrate.

The study of the interaction of the substrate with the enzyme is a key step in the comprehension of the structure-function relationship in peroxidases. This study can be successfully accomplished through NMR spectroscopy, once the assignment of most of the signals of residues in the active site is available.[1] The latter has been obtained through a large variety of NMR experiments which include 2D NOESY, COSY and TOCSY maps, 2D ^{1}H-^{13}C HETCOR as well as 2D edited NOESY experiments.[2-5]

We will present here the characterization of the interaction of small aromatic molecules as p-cresol, veratryl alcohol and phenols with peroxidases. The interaction with mediators like Mn(II) for MnP and veratryl alcohol for LiP are also presented. The Mn(II) binding site has been investigated through the study of the interaction of the protein with several metal ions, including Mn(II) itself and Co(II). Finally, the binding sites for the Ca(II) ions and their role in determining the protein structure will be discussed.

1. Banci, L.; Bertini, I.; Bini, T.; Tien, M.; Turano, P. *Biochemistry* **1993**, *32*, 5825.
2. Banci, L.; Bertini, I.; Turano, P.; Tien, M.; Kirk, T.K. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 6956.
3. Banci, L.; Bertini, I.; Kuan, I-C.; Tien, M.; Turano, P.; Vila, A.J. *Biochemistry* **1993**, *32*, 13483.
4. Banci, L.; Bertini, I.; Pease, E.; Tien, M.; Turano, P. *Biochemistry* **1992**, *31*, 10009.
5. Banci, L.; Bertini, I.; Pierattelli, R.; Vila, A.J. *Inorg. Chem.* **1994**, in press.

Molecular Dynamics Studies on Cu, Zn Superoxide Dismutase

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Copper, zinc superoxide dismutase (SOD) is a dimeric enzyme containing a copper and a zinc ion in each subunit[1]. The copper ion, which is essential to the enzyme activity, is located at the bottom of a shallow cavity in the protein. The surface of this cavity bears numerous charged residues that are believed to play an important role in the catalytic mechanism by electrostatically steering the substrate to the copper site.

The physiological function of SOD is the dismutation of the superoxide radical anions. The rates for the catalytic process are very high ($2 - 3 \times 10^9 \text{s}^{-1}\text{M}^{-1}$ at 298 K) and are diffusion-controlled.

Molecular dynamics (MD) calculations can be of great help in understanding the structure and function of this protein[2].

We have performed MD studies on wild type SOD and some mutants to elucidate the role of important aminoacids present in the cavity[3]. We have learnt that the enzyme activity, beside by strong electrostatic effects, is influenced by the width and the dynamics of the active site channel. Indeed, the decrease in enzymatic activity observed for some mutants on Arg 143 correlates with the width of the active site channel. Furthermore, we have seen that the conformation of some aminoacids facing the active channel, forming the so called "Electrostatic Loop VII", are important in determining the correct local electrostatic field which should guide the substrate to the reaction center. We have also characterized the dynamic behavior of the ordered water molecules located in the active site.

We have recently performed MD calculations on the monomeric form of SOD[4], obtained in our laboratory[5] in order to investigate the structural changes upon removal of one subunit.

[1] Tainer, J. A.; Getzoff, E. D.; Beem, K. M.; Richardson, J. S .; Richardson, D. C., J. Mol. Biol., 1982, 160, 181-217.

[2] McCammon, J.A., Harvey, S., Dynamics of Proteins and Nucleic Acids, Cambridge University Press: Cambridge, 1987.

[3] (a) Banci, L.; Carloni, P.; La Penna, G.; Orioli, P.,J. Am. Chem. Soc., 1992,114,6994-7001 (b) Banci, L.; Carloni, P.; Orioli, P., Proteins: Struc. Funct. Gen., 1994, 18,216-230.

[4] Banci, L.; Carloni, P.; Orioli, P., in preparation.

[5] Bertini, I., Chiu, C.Y., Mullenbach, G.T., Viezzoli, M.S. Eur. J. Biophys., in press.

SYNTHETIC ANALOGUES OF NITROGENASE COFACTOR (FeMoco): THE MOLYBDENUM COORDINATION ENVIRONMENT.

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At the end of 1992, Kim and Rees[1] proposed a structure for the iron-molybdenum cofactor (FeMoco) of the molibdenum nitrogenase from *Azotobacter vinelandii*. Modelling the electron density of the cluster, as found in X-ray crystallography studies, suggested FeMoco to have an extended iron-sulfur cluster framework, of stoichiometry Fe_7MoS_9(homocitrate).

The cluster is of a complexity that direct synthesis of a chemical analogue is inappropriate; rather fragments of the cluster are synthesised and their properties investigated. Here we describe the preparation of analogues with a molybdenum coordination environment similar to that in the model compound.

Reaction of the known cluster $[MoFe_3S_4(SEt)_3(Cl_4cat)(solv)]^{2-}$ (cat=catechol) or of novel cluster $[MoFe_3S_4(L1)(Cl_4cat)(solv)]^{2-}$ (H_3L1=1,4,7-tris(p-mercaptobenzoyl)-1,4,7-triazacyclononane)[2] with imidazole or with histidine methyl ester forms clusters with the desired molybdenum coordination environment, NO_2S_3. The Fe_3S_4 unit acts as a tridentate sulfur ligand. The ligand "L1" extends further the coordination sphere to include three, non-exchangeable, distant sulfur atoms. ^{1}H-NMR and Mössbauer spectroscopic data are presented.

1.   J.Kim, D.C.Rees, Science, **257**, 1677 (1992); M.K.Chan, J.Kim, D.C.Rees, Science, **260**, 792 (1993).

2.   D.J.Evans, G.García, G.J.Leigh, M.S.Newton, M.D.Santana, J. Chen. Soc., Dalton Trans., 3229 (1992).

PLATINATED HUMAN SERUM TRANSFERRIN

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We are investigating the use of transferrin (TF, 80 kDa) for delivering platinum anticancer complexes to tumor cells via their surface receptors for this protein. We have used a combination of ^{1}H, [^{1}H,^{15}N] and [^{1}H,^{13}C] NMR spectroscopy to show that cis-[PtCl$_2$(NH$_3$)$_2$] and [Pt(1,2-diaminoethane)Cl$_2$] platinate specific methionine residues in intact TF and its recombinant N-lobe (40 kDa), and have studied the kinetics of binding. A remarkable finding is that [^{1}H,^{15}N] cross-peaks for Pt am(m)ines are well resolved for TF and its N-lobe despite their large sizes (for NMR studies). Platination did not inhibit receptor binding or cell uptake of TF.

ADDUCTS OF THE ANTICANCER DRUG CARBOPLATIN

("PARAPLATIN") WITH SULFUR AMINO ACIDS:

DETECTION IN URINE.

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We are investigating the role of sulfur amino acids in the metabolism of platinum anticancer drugs [1,2]. Not only may they be involved with the detoxification process, but also in the transfer of Pt onto DNA bases.

Reactions of carboplatin with thiol containing amino acids (N-acetyl-*L*-cysteine, and glutathione) are very slow (k~10^{-9} $M^{-1}s^{-1}$) and result in thiolate bridged species, whereas the reaction with *L*-methionine is much faster (k=2.7×10^{-3} $M^{-1}s^{-1}$ 298K) and involves the ring- opened species [Pt(CBDCA-*O*)(NH$_3$)$_2$(*L*-methionine-*S*)] as a stable intermediate.

Using[^{1}H,^{15}N] NMR spectroscopy and 15N-labelled carboplatin, [Pt(CBDCA-*O*,*O*)(^{15}NH$_3$)$_2$], we have observed similar adducts in the urine of mice treated with the drug.

1. Murdoch, P. d. S.; Ranford, J.D.; Sadler, P.J.; Berners-Price, S.J. *Inorg. Chem.* **1993**, *32*, 2249

2. Barnham, K.J.; Djuran, M.I.; Murdoch, P. d. S.; Sadler, P.J. *J. Chem. Soc. Chem. Commun.* **1994**, 721

INTRAMOLECULAR H-BONDING IN Pt(II) and Pd(II)

MONONUCLEOTIDE COMPLEXES: NMR AND X-RAY STUDIES

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We are studying intramolecular NH-5′-phosphate H-bonding in Pd(II) and Pt(II)

mononucleotide complexes in solution using [^{1}H,^{15}N] and 1D NOE difference NMR

spectroscopy [1]. We also report the X-ray crystal structure of [Pd(en)(5′-GMP)$_2$].9H$_2$O, which

appears to provide the first example of such H-bonding in a crystalline mononucleotide complex.

The structure shows isolated molecules with head-to-tail configuration of the bases, an *anti*

conformation about the glycosidic bond, and *endo* sugar conformation. Pseudo-octahedral H$_2$O

molecules play key roles in the H-bonding pattern. We will also report the X-ray structure of the

analogous Pt(II) complex.

1. Berners-Price, S.J.; Frey, U.; Ranford, J.D.; Sadler, P.J. *J. Am. Chem. Soc.* **1993**, *115*, 8649

Observation of an Fe(III) - tyrosinate and a tyrosine radical during ferritin-catalysed Fe(II) oxidation. Do these species play an essential role in ferritin H chain ferroxidase activity ?

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Iron storage in ferritin involves Fe(II) uptake into the protein shell, Fe(II) oxidation at ferroxidase centres on H chains, Fe(III) migration, nucleation of ferrihydrite iron-cores and Fe(II) oxidation on iron-core surfaces. Here we are concerned with the elucidation of the mechanism of Fe(II) oxidation with the aid of stopped-flow kinetics and EPR spectroscopy.

Two Fe(III) species are observed during the early stages of Fe(II) oxidation by recombinant human H chain homopolymer (rHuHF) : an Fe(III) μ-oxo-bridged dimer (identified by Mössbauer spectroscopy) and an Fe(III)-tyrosinate (absorbing at 550nm). The 550nm absorbance rises to a maximum at 0.2 secs and then decays, whilst other oxidation products continue to rise. The Fe(III)-tyrosinate seems to account for only a small fraction of the Fe(II) oxidised. It is not observed in site-directed variants in which ferroxidase centre amino acid residues have been substituted, including Y34F. Fe(II) oxidation is nevertheless catalysed in Y34F (more slowly). *E.coli* bacterioferritin gives no Fe(III)-tyrosinate, even though oxidation is fast.

Two types of EPR radical signals have been observed during Fe(II) oxidation, one with fine structure, the other without. The latter decays and the former becomes more evident over 3 min. The fine structure signal has a g-factor of 2.0072 and a hyperfine splitting of 7-8 G. A similar fine structure signal, which is attributed to a tyrosine radical, is also seen with horse spleen ferritin. The rHuHF variant Y34F shows only a weak EPR signal lacking fine structure, consistent with the rHuHF radical's being centred on Tyr-34. No ^{17}O hyperfine couplings are observed when $^{17}O_2$ is employed as an oxidant, indicating that none of the signals is from oxy radicals. Radical signals are enhanced 3-4 fold when H_2O_2 replaces O_2 as oxidant, but their yields account for only one radical per 2-3 protein molecules or per 300-500 Fe(II) oxidised.

What are the roles of the Fe(III)-tyrosinate and the tyrosine radical in ferritin-catalysed Fe(II) oxidation ? The radicals appear to be byproducts due to Fenton Chemistry. The Fe(III)-tyrosinate may also be a byproduct, but its role is at present obscure.

Extent of Intramolecular Aromatic-Ring Stacking in Ternary Cu^{2+} Complexes Formed with Flavin Mononucleotide (FMN^{2-}) and 2,2'-Bipyridyl (Bpy) or 1,10-Phenanthroline (Phen)

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Flavoenzymes, often being metal ion-dependent, catalyze redox reactions[1] via the 7,8-dimethylisoalloxazine (dmia) residue and there is evidence that charge-transfer or stacking interactions are important. FMN^{2-} (= F), also known as riboflavin 5'-phosphate, is one of the flavo-coenzymes.
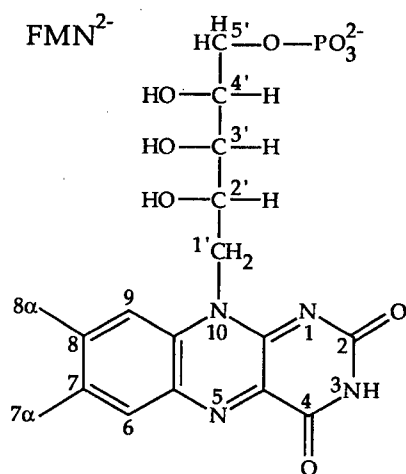
Its self-stacking tendency can be quantified via ^{1}H-NMR measurements and the data may be explained with the isodesmic model[2] for an indefinite non-cooperative self-association with equal equilibrium constants for the various steps (eq 1):
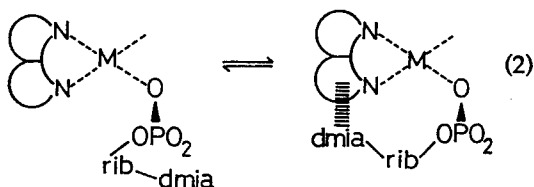
$$(F)_n + F \rightleftharpoons (F)_{n+1}$$
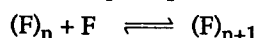
$$K_{St} = [(F)_{n+1}]/[(F)_n][F] \qquad (1)$$

The intramolecular equilibrium (2) also arises from stacking interactions, namely between the aromatic rings of Bpy or Phen and the dmia residue of FMN^{2-} in the ternary Cu(Bpy/Phen)(FMN) complexes. The stability of the Cu(Bpy)(FMN) complex (log $K_{Cu(Bpy)(FMN)}^{Cu(Bpy)}$ = 3.56 ± 0.02/3 σ) is by 0.68 ± 0.07 log unit larger than expected for a simple $Cu(Bpy)^{2+}$-phosphate[3] interaction. In contrast, the stability of the 'model' Cu(Bpy)(glycerol-1-phosphate) complex[4] corresponds exactly to a sole coordination of $Cu(Bpy)^{2+}$ to the $-PO_3^{2-}$ group, confirming thus that the increased stability of Cu(Bpy)(FMN) is indeed due to intramolecular stacking. The formation degree of its stack is 79 ± 3% (eq 2) whereas that of Cu(Phen)(FMN), as expected, is somewhat higher, namely 90 ± 2%.

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(1) S. Ghisla and V. Massey, *Eur. J. Biochem. 181*, 1-17 (1989).
(2) N. A. Corfù, R. Tribolet, and H. Sigel, *Eur. J. Biochem. 191*, 721-735 (1990).
(3) D. Chen, M. Bastian, F. Gregáň, A. Holý, and H. Sigel, J.C.S. Dalton T., 1537-1546 (1993).
(4) G. Liang, D. Chen, M. Bastian, and H. Sigel, *J. Am. Chem. Soc. 114*, 7780-7785 (1992).

An electrochemical and spectroscopic survey on cytochrome c_2 from *Rps. palustris.*

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Cytochrome c_2 from *Rhodopseudomonas palustris* is a typical class I cytochrome, whose molecular weight (13500 Daltons) is somewhat larger than that of mitochondrial cytochromes c, while its redox potential is around 350 mV, about 100 mV more positive than that of horse heart cytochrome c [1]. Given the great similarities between bacterial cytochromes c_2 and the mitochondrial ones, this difference is quite relevant. Therefore, much work has been done in the past years in order to determine the structural and physico-chemical bases for this difference [2].

Cytochrome c_2 from *Rps. palustris* has been purified [3] and studied through 1D and 2D ^{1}H NMR spectroscopy and cyclic voltammetry.

The dependence of the redox potential as a function of the ionic strength in the presence of different anions and of the pH was followed through cyclic voltammetry. The increase in ionic strength caused a decrease of the redox potential. The behavior in presence of ClO_4^- and HCO_3^- suggests the existence of specific sites of interaction for these anions on the protein surface, that lack for Cl^- .

The pH-dependence of the redox potential indicated the presence of two different equilibria. The former acid-base equilibrium has a pK_a around 6.9, that is very similar to those measured in other cytochromes c_2 [2], and is due to the deprotonation of a residue, most probably the non-coordina ting histidine 53. The latter equilibrium is indicative of the existence of two pH-dependent states, one stable at neutral pH and the other typical of alkaline pH. These two species have quite different redox potentials (350 mV and -55 mV respectively), hence it has been possible to quantitatively follow their interconversion and to determine a pK_a value around 9. The NMR data on ferricytochrome c_2 showed that this transition involves the displacement of the axial methionine and causes a distortion of the geometry of the metal

chromophore. At present it is not known whether this pH-dependent transition, that is common to the mitochondrial cytochromes [2], has a physiological relevance or not. Extensive NMR work is underway in order to obtain a better insight into the structure of this interesting molecule.

The present data demonstrate clearly that the combination of NMR spectroscopy and electrochemical techniques constitutes a particularly suitable tool to study electron transfer proteins and to analyse their properties.

1. Bartsch R. G.; in *The Photosynthetic Bacteria*, Plenum Press, New York, 1976, 249-279.
2. Moore G. R., Pettigrew G. W.; *Cytochromes c*, Springer-Verlag, Berlin Heidelberg, 1990.
3. Bartsch R. G.; *Methods in Enzymology*, **1971**, 23, 344-363.

MAGNETIC RESONANCE FINGERPRINTS OF FeS CLUSTERS: ISOLATION AND CHARACTERIZATION OF A Fe_7S_8 FERREDOXIN FROM *RPS. PALUSTRIS*.

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A previously unknown ferredoxin has been isolated and purified from anaerobic growth of the photosynthetic bacterium *Rps. palustris*. The isolated protein gives a single band on SDS-PAGE. Gel-filtration chromatography shows that the protein is monomeric with a molecular weight of about 19,000 Daltons. The protein contains 7.7-8.4 iron atoms/mole and its ε_{400} is around 20,000 $M^{-1}cm^{-1}$.

Cyclic voltammetric experiment gives clear indication of two redox steps, one around -260 mV and the other around -560 mV versus NHE. Electrochemical irreversibility precludes a more precise determination. Chemical reduction to the half-reduced species can be achieved with $Na_2S_2O_4$, whereas the second reduction step can only be obtained with $NaBH_4$. EPR and NMR experiments on the three redox states provide a clear-cut identification of a [3Fe-4S] and a [4Fe-4S] clusters [1,2].

Recent data on a ferredoxin from the microbiologically close *Rps. rutila* [3] can be interpreted in the light of the present findings as evidence for a dimeric 7Fe-8S protein whose subunit has a molecular weight similar to the ferredoxin from *Rps. palustris*.

1   Nagayama K., Ohmori D., Imai T., Oshima T. (1983) FEBS Lett. **158**, 208-212.

2   Bertini I., Ciurli S., Luchinat C. Angew. Chem., in press.

3   Meyer T. E., Fitch J., Van Driessche G., Van Beeumen J., Fischer U., Bartsch R. G., Cusanovich M. A. (1991) Arch. Biochem. Biophys. **286**, 389-393.

TRANSFERRIN AS A LIGAND OF NON-IRON METALS.

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The conformation in solution of three different metal(III) transferrins, namely aluminum, gallium and indium transferrin, has been investigated by absorption, CD, ^{1}H NMR and ^{13}C NMR spectroscopies. The formation of the respective metal-transferrin complexes and the characteristic 2:1 metal-to-protein binding stoichiometry were unambiguously demonstrated, in all cases, through UV difference studies. The ^{13}C NMR spectra of these metallotransferrins are very similar to one another pointing out that the arrangement of the synergistic anion in the binding site must be essentially the same. However, the CD spectra in the UV reveal the occurrence of significant differences between indium transferrin, on one side, and the other two derivatives, on the other. Also, the ^{1}H NMR spectra exhibit a number of different features suggesting the occurrence of metal-induced conformational heterogeneity. We suggest that metal induced conformational heterogeneity may affect the recognition process of these metallotransferrins at the level of the cell surface transferrin-receptor and result in a different metabolic fate of these metals in the organisms. Extensive binding studies of the above metallotransferrins with the transferrin receptor are now needed to support these ideas.

# COBALT(II) SUBSTITUTION IN THE THREE-ZINC METAL SITE OF NUCLEASE P1

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Nuclease P1 (EC 3.1.3.6) from the mold *Penicillum citrinum* is a phosphate ester hydrolyzing enzyme (MW = 36 kDa) which specifically cleaves single-stranded RNA and DNA into 5'-mononucleotides, and 3'-monophospho-nucleotides to nucleosides plus phosphate.[1] The X-ray structure at 2.8 Å resolution[2] shows that the metal site contains three clustered zinc atoms at the bottom of a substrate binding cleft: two metals are connected by a bidentate bridging aspartate residue and a water molecule, originating a dinuclear core, while an individual zinc atom lies 4.7 and 5.8 Å from the members of the above pair. Histidine and aspartate residues plus water molecules and the main chain N and O atoms of a triptophan residue bind the metals as shown in figure:[2]
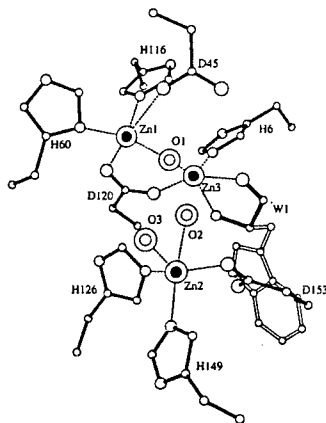
All the zinc atoms display a distorted trigonal bipyramidal five-coordination. It is thought that the mononuclear zinc site is directly involved in catalysis, while the dinuclear core should assist the process (possibly activating the substrate) and exert a structural role.[2] The understanding of the individual roles of the metals and the aminoacidic residues of this polymetallic site (belonging to the class of the so-called "cocatalytic" zinc sites)[3] is a frontier issue in the

metallobiochemistry of zinc enzymes. Substitution of one or more zinc atoms with spectroscopically active Co(II) would allow monitoring changes upon binding of exogenous molecules, hence would be a source of valuable mechanistic information. We succeeded in loading one Co(II) ion in place of the most EDTA-labile zinc atom of nuclease P1. The electronic spectra indicate an octahedral metal coordination, consistent with the overall appearance of the hyperfine-shifted ^{1}H NMR resonances arising from the protons in the surroundings of the metal. The ^{1}H NMR data are being analyzed in order to identify which metal has been substituted, and its role in the interaction with substrate analogs and inhibitors.

REFERENCES

1. Fujimoto, M.; Kuninaka, A,; Yoshino, H. *Agr. Biol. Chem.*, **1974**, *38*, 1555-1561.
2. Volbeda, A.; Lahm, A.; Sakiyama, F.; Suck, D. *EMBO J.* **1991**, *10*, 1607-1618.
3. Vallee, B. J.; Auld, D. S. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2715-2718.

Oxygen-stable cob(II)alamin is not involved in catalysis of coenzyme B_{12}-dependent 2-methyleneglutarate mutase from *Clostridium barkeri.*

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2-Methyleneglutarate mutase catalyzes the reversible adenosylcobalamin-dependent carbon skeleton rearrangement of 2-methyleneglutarate to (R)-3-methylitaconate in the fermentation of nicotinate to ammonia, acetate, propionate and carbon dioxide by the strict anaerobic bacterium *Clostridium barkeri* [1]. The purified clostridial enzyme (molecular mass = 300.000 Da) is a homotetramer containing adenosylcobalamin (coenzyme B_{12}) and varying amounts of cob(II)alamin (6-11%). Interestingly, the cob(II)alamin bound to the mutase was not sensitive towards oxygen and showed an EPR-Signal ($g_{x,y}$ = 2.24) [2] . Only about half of the amount of the cobalamins could be removed by treatment with 8 M urea. Thereby the enzymatic activity was totally lost; it could be regenerated by mere addition of adenosylcobalamin. No attempts were successful in complete removal of the coenzyme from the holoenzyme [3]. Incubation of 2-methyleneglutarate mutase with its substrate induced a second EPR-signal that overlapped with that of the oxygen-stable cob(II)alamin [2].

In order to investigate the role of cob(II)alamin in the mutase we cloned the gene *mgm* encoding 2-methyleneglutarate mutase and expressed it in *E. coli* (10-12% of total cellular protein). A three step purification procedure led to a completely cobalamin-free, inactive apoenzyme which could be activated by mere addition of adenosylcobalamin. The resulting specific activity was twice as high as that of the mutase purified from *C. barkeri.*

UV/VIS and EPR-spectra did not indicate the presence of cob(II)alamin or aquocobalamin. After addition of substrate, a paramagnetic species was generated. Its EPR-spectrum was of the same type as observed with related glutamate mutase ($g_{x,y}$ = 2,13 and g_z = 1,98) and may be regarded as a sixfold coordinated cob(II)alamin [5]. After aerobic as well as after anaerobic incubation over night with the substrate, a second EPR-signal appeared which could represent the cob(II)alamin that is observed with the clostridial enzyme even in the absence of substrate. These results show that the oxygen-stable cob(II)alamin is not involved in catalysis and probably represents an inactive 2-methyleneglutarate mutase in *C. barkeri* which is generated after several turnover even *in vivo.*

Ref.: [1] Kung, H.F., Cederbaum, S., Tsai, L. & Stadtman, T.C. (1970) *Proc. Natl. Acad. Sci. USA* , **65**, 978-984.
[2] Michel, C., Albracht, S.P.J. & Buckel, W. (1992) *Eur. J. Biochem.*, **205**, 767-773
[3] Zelder, O. & Buckel, W. (1993) *Biol. Chem. Hoppe-Seyler*, **374**, 85-90
[4] Beatrix, B., Zelder, O., Linder, D. & Buckel, W. (1994) *Eur. J. Biochem.*, **221**, 101-109
[5] Leutbecher, U., Albracht, S.P.J. & Buckel, W. (1992) *FEBS Lett.*, **307**, 144-146.

SPECIFIC NMR ASSIGNMENTS FOR HUMAN SERUM TRANSFERRIN BY STABLE ISOTOPE LABELING

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Human serum transferrin is an iron binding protein, with a molecular mass of 80 kDa, and a bilobal structure. Previously we have used high resolution ^1H NMR spectroscopy to investigate structural changes which accompany metal (Fe^{3+}, Ga^{3+}, Al^{3+}) and anion (HCO_3^-, oxalate) binding [1,2]. We have now labelled this protein with ^{13}C and ^{15}N, both at single sites, and uniformly throughout the N-lobe. Stable isotope labeling has proved to be an excellent method of determining solution structures of proteins containing fewer than 200 amino acids, but for proteins larger than this, problems occur as the line widths increase. We show here, however, that it is possible, by using multidimensional NMR, to specifically assign residues, both for the whole protein and for the N-terminal half molecule. We have assigned residues around the iron binding site, and this has allowed us to examine in detail the binding of a number of different metals and anions.

1. Kubal, G.; Mason, A.B.; Patel, S.U., Sadler, P.J.; Woodworth, R.C. *Biochemistry* **1993**, *32*, 3387

2. Kubal, G.; Mason, A.B.; Sadler, P.J., Tucker, A.; Woodworth, R.C. *Biochem. J.* **1992**, *285*, 711

An Imidazolate-bridged Heterobinuclear (Copper-Zinc) Complex of a Macrobicyclic Ligand, Model of the Active Site of Superoxide Dismutase

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Copper-zinc superoxide dismutase contains in its active site an imidazolate bridged bimetallic center (one copper(II) and one zinc(II) atom) . Several imidazolate-bridged dicopper complexes have been described with open-chain or monocyclic ligands and the pioneering work of S.J. Lippard in this field must be emphazised . A few imidazolate-bridged heterobinuclear Cu-Zn complexes have been described.

We describe here, an imidazolate-bridged copper-zinc complex $[LCuZnIm]^{3+}$ of a unique macrobicyclic ligand L. The complex was characterized by elemental analysis, FAB^+ mass spectrum
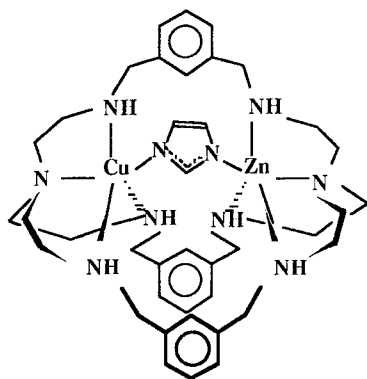
and by X-ray diffraction technique (fig.). ThisCu-Zn distance is shorter than in SOD (6 Å instead 6.3 Å) .The electronic spectrum in aqueous solution of $[LZnCuIm](ClO_4)_3$ and the EPR spectrum for a 10^{-3} M frozen solution (100 K) of $[LZnCuIm](ClO_4)_3$ in water:DMSO 1:1 (v:v) were studied and suggested a trigonal bipyramid geometry.

The complex-catalysed dismutation of superoxide anion (IC_{50} value, 0.5 μM evaluated by the NBT assay) survives in the presence of bovine serum albumine .

References: *J. Chem. Soc. Chem. Commun.,* 1994, in press

Magnetic And UV/VIS-Spectroscopic Investigations On Ferromagnetic Coupled Copper(II)-Models

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Mixed bridged multinuclear metal centers with exchange coupling via oxo/carboxylato ligands are very common in metalloproteins like hemerythrin, purple acid phosphatases or ribonucleotid reductase. Since the discovery of a trimeric copper(II)- unit in ascorbat- oxidase the interest in trinuclear complexes as model- systems for active sites of metalloproteins is increasing.

In this contribution results of magnetization measurements especially on one trimeric copper(II) compound with ferromagnetic exchange coupling (Figure 1) are presented. Additionally to this we would like to show results of UV/VIS-spectroscopic investigations.
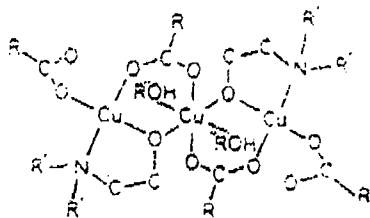
(Figure 1)

Literature:

1) Z. Tomkowicz, P. Fleischhauer, W. Haase, M. Baran, R. Szymczak and A. J. Zaleski, J. Magn. Magn. Mater. 127, L11 (1993).
2) S. Gehring, P. Fleischhauer, H. Paulus and W.Haase, Inorg. Chem. 32, 54 (1993).

FORMATION OF A SUBSTRATE ANALOG RADICAL INTERMEDIATE IN THE MOUSE RIBONUCLEOTIDE REDUCTASE REACTION

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Ribonucleotide reductase is an essential enzyme in all living organisms. It catalyzes the reduction of the four common ribonucleotide diphosphates to the corresponding deoxyribonucleotides and provides the cell with the proper supply of precursors for DNA synthesis. The holoenzyme consist of two subunits, a large one called protein R1 and a smaller one called protein R2. Each of the subunits consists of two identical polypeptide chain. Each R2 polypeptide contains a pair of high spin ferric ions , antiferromagnetically coupled through a μ-oxo bridge, and a tyrosyl free radical essential for activity. The allosterically regulated protein R1 contains the redox-active sulfhydryl groups. The active site of the enzyme includes parts from both subunits.

A transient free radical has previously been observed in the E. coli ribonucleotide reductase reaction with the suicide inhibitor 2´-azido-2´-deoxycytidine 5´-diphosphate. It was shown to be derived from the azido moiety of the inhibitor, but its exact nature has not been established (1,2). The observation of this radical is the only direct evidence to date of the ability of the enzyme to catalyse the formation of a substrate related free radical. We have now observed a similar transient free radical in the reaction of the mouse ribonucleotide reductase with 2´-azido-2´-deoxycytidine 5´-diphosphate. The characteristics of the new free radical and its EPR signal will be presented.

References:

1.   Sjöberg, B.-M., Gräslund, A. and Eckstein, F. (1983) J. Biol. Chem. 258, 8060-8067.
2.   Stubbe, J. (1990) Adv. Enzymology (ed. A. Meister) 63, 349-419.

EXCHANGE INTERACTIONS IN TETRAMERIC CLUSTERS

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Exchange interactions determine spectroscopic and magnetic properties of polinuclear clusters of transition ions in biological systems. The problem of the Heisenberg exchange was solved in general form for the tetrameric $[Fe_4S_4]$ clusters of ferredoxins, enzymes, high-potential proteins, their synthetic models, and for mixed valence Mn_4 clusters of the active center of Photosystem II. The Hamiltonian $H_A = 2[J_{12}s_1s_2 + J_a(s_1s_3 + s_2s_3) + J_b(s_1s_4 + s_2s_4) + J_{34}s_3s_4]$ describes the systems with the mixing of the S_{34} intermediate spins. The Heisenberg model $H_B = -2[J_{12}s_1s_2 + J_\alpha(s_1s_3 + s_1s_4) + J_\beta(s_2s_3 + s_2s_4) + J_{34}s_3s_4]$ describes the clusters with the mixing of the states with different S_{12} (S_{34}, S are fixed). These models have analytical solutions for the states with S=1/2 and are the generalization of the Kambe approach for the tetramers with fixed intermediate spins. The exchange matrices for the general Heisenberg tetrameric model $H = -2[J_{12}s_1s_2 + J_{13}s_1s_3 + J_{23}s_2s_3 + J_{14}s_1s_4 + J_{24}s_2s_4 + J_{34}s_3s_4]$ were obtained by using the technique of irreducible tensor operators. The states of the general model are characterized by the total spin S and by the mixture of the intermediate spins. This mixture leads to the exchange variation of the $K_i = <s_{iz}>/<S_z>$ coefficients, which determine the correlation $g_{eff} = \sum K_i g_i$ between the cluster (g_{eff}) and local (g_i) g-factors, and also the correlation $A_i = K_i a_i$ between the effective A_i and local a_i hyperfine constants. The exchange dependence of g_{eff} explains observed values $g_{eff} < 2$ for reduced ferredoxin $[Fe_3(II)Fe(III)]$ clusters and $g_{eff} > 2$ for oxidized high-potential protein $[Fe_3(III) Fe(II)]$ clusters. The exchange dependence of the A_i explains signs and magnitudes of the effective hyperfine constants observed in Mössbauer and ENDOR experiments, the modification of the effective hyperfine constants under the cluster distortions. Double exchange interaction between two mixed-valence iron atoms in tetramer results in the formation of the exchange-resonance states. In the Anderson-Hasegawa model of double exchange, strong double exchange in mixed-valence dimers leads to ferromagnetic ground state with maximal total spin. In mixed-valence iron tetramers, strong double exchange between two ions leads to antiferromagnetic ground state with S=1/2.

In the mixed-valence manganese tetramers of Photosystem II, the mixing of the states with different intermediate spins determines the hyperfine structure of the EPR signal.

EXCHANGE VARIATION OF HYPERFINE CHARACTERISTICS
OF TETRAMERIC MANGANESE CLUSTERS OF
S_2 CENTER OF PHOTOSYSTEM II

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Tetrameric $[Mn_3(III)Mn(IV)]$ and $[Mn_3(IV)Mn(III)]$ clusters are the prototypes of the Mn cluster of the active center of Photosystem II in the S_2 state. The exchange interactions in the Mn tetramers are analyzed in the generalized model $H_B = -2[J_{12}s_1s_2 + J_\alpha(s_1s_3 + s_1s_4) + J_\beta(s_2s_3 + s_2s_4) + J_{34}s_3s_4]$ and in general Heisenberg model $H_G = -2[J_{12}s_1s_2 + J_{13}s_1s_3 + J_{23}s_2s_3 + J_{14}s_1s_4 + J_{24}s_2s_4 + J_{34}s_3s_4]$. The levels of the H_B model are characterized by the mixture of the S_{12} intermediate spins. The levels of the general Heisenberg model H_G are characterized by the total spin S, all intermediate spins are mixed. In the H_B model, the spin coefficients $K_i=<s_{iz}>/<S_z>$, which determine the correlation $A_i=K_ia_i$ between the effective A_i and local a_i hyperfine constants, have the following analytical form for all Mn atoms of the cluster

$[Mn_3(III)Mn(IV)]$: $K_1=[1-(2j+25\Delta)/\Gamma_1]/3$, $K_2=[1-(2j-23\Delta)/\Gamma_1]/3$,
$K_3=2[1+(4j+2\Delta)/\Gamma_1]/3$, $K_4=-[1+(4j+2\Delta)/\Gamma_1]/3$;

$[Mn_3(IV)Mn(III)]$: $K_1=[1-(j+8\Delta)/\Gamma_2]/3$, $K_2=[1-(j-7\Delta)/\Gamma_2]/3$,
$K_3=-[1+(2j+\Delta)/\Gamma_2]/3$, $K_4=2[1+(2j+\Delta)/\Gamma_2]/3$,

where $j=J_\alpha-J_{12}$, $\Delta=J_\alpha-J_\beta$, $\Gamma_1=\sqrt{4j+4j\Delta+25\Delta}$, $\Gamma_2=\sqrt{j+j\Delta+4\Delta}$, $\Sigma K_i=1$. The exchange modification of the K_i coefficients leads to exchange variation of the hyperfine structure of the EPR signal with g=2.

The complex Mn_4 systems with the trapezoid, bent, "butterfly", T-type, distorted tetrahedron structures cannot be described in the Kambe models with fixed intermediate spins or in the model with mixing of one intermediate spin. The spin states and hyperfine characteristics of these complex Mn clusters are described in the general Heisenberg model H_G with mixing of all intermediate spins. The variation of the exchange interactions change the interline space, the number and intensities of hyperfine lines.

The model of the exchange variation of the hyperfine structure allows one to consider the correlation between the concrete form of the Mn_4 exchange cluster and observed hyperfine structure of the EPR signal.

The electronic structure of the $[Fe_4S_4]^{3+}$ cluster in high potential iron-sulfur proteins

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High-potential proteins (HiPIP) may be involved in the electron-transfer chain in some photosynthetic bacteria. Mössbauer, EPR, and ENDOR data show that the $S=1/2$ ground state is formed by antiferromagnetic exchange interactions among the iron ions spins $s_1(Fe^{3+})=s_2(Fe^{3+}) = s_3(Fe^{3+})=5/2$ and $s_4(Fe^{2+})=2$. The usual Heisenberg model considers the HiPIP cluster as made up by two interacting pairs: the ferric pair s_1, s_2 and the mixed valence pair s_3,s_4. The spin Hamiltonian has the form $H=J\Sigma s_i s_j + \Delta J_{12}s_1s_2 + \Delta J_{34}s_3s_4 + BT_{34}$ and the energy levels are characterized by the fixed intermediate spins of the pairs. This model of the $[Fe_3(III)Fe(II)]$ cluster with a $|(S_{12}=4, S_{34}=9/2) S=1/2>$ ground state qualitatively describes the main features of the system (opposite singes of the effective hyperfine constants, $S=1/2$ ground state, $g_{eff}>2$). However, the $|(S_{12}=4, S_{34}=9/2)S=1/2>$ level leads to an overestimate of the values of the effective hyperfine constants. In the same frame of C_{2v} symmetry, the $|(S_{12}=3, S_{34}=7/2)S=1/2>$ ground state provides a better agreement with experimental hyperfine constants. A strong double exchange is necessary to obtain this ground state.

We have considered an alternate descripton of the coupling interaction in the $[Fe_3(III)Fe(II)]$ cluster. The proposed model describes the HiPIP cluster in the oxidized state as a slightly distorted trigonal system. The ferric-ferric interactions in the triangle between the Fe(III) ions are sizeblyb stronger than their interactions with the Fe(II) ion. The slightly distorted (compressed) exchange tetrahedron with double exchange in the mixed-valence "34" pair gives effective hyperfine constants $A_1=A_2=21.5$, $A_{3,4}^{av}=-33.5$, $g_{eff}=2.066$. These quantities are in a good agreement with experimentally observed $A_{1,2}=21.4\pm1.5$, $A_{3,4}=-31.5\pm1$ and $g_{eff}=2.07$ for HiPIP II from E.halophila. The spin ground state of the $[Fe_3(III)Fe(II)]$ cluster with exchange parameters $J_{34}=220$ cm^{-1} , $J_{14}=J_{24}=240$ cm^{-1}, $J_{13}=J_{23}=500$ cm^{-1} and $J_{12}=470$ cm^{-1} is described by the wavefunction $\Phi_{gr}=0.94|(4,9/2)1/2> + 0.32|(4,7/2)1/2>$. Double exchange results in the averaging of the hyperfine characteristics of ions 3 and 4, however it does not destroy the ground state originated from the Heisenberg interactions. These calculations demonstrate the strong influence of the exchange interactions on the spectroscopic properties of the HiPIP clusters.

THE OXIDATION OF HEMOCYANIN: KINETICS, REACTION MECHANISM AND CHARACTERISATION OF THE MET-HEMOCYANIN PRODUCT.

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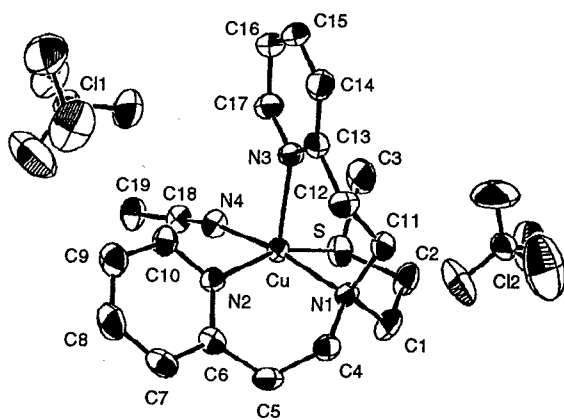
The active site of oxy-hemocyanin (oxy-Hc) has been described as a dinuclear Cu(II) complex with a peroxide bound as a $\mu: \eta^2\text{-}\eta^2$ peroxo bridge. Oxy-Hc reacts at acidic pH with a number of relatively weak acids and generates an EPR silent met-form which contains a dinuclear cupric complex. The kinetics of the reaction has been studied with *Octopus vulgaris* Hc in the presence of fluoride or azide and under different experimental conditions of pH, oxygen partial pressure, temperature. In the absence of oxygen, the deoxy-Hc (present as a dinuclear Cu(I) site) did not react neither with fluoride nor with azide, demostrating that oxy-Hc is the active species. The reaction on oxy-Hc did not follow a pseudo-first order kinetics. The initial reaction rate increased as the temperature was raised from 20° to 37° C. The reaction of oxy-Hc was almost negligible at pH 8 but it increased on going to pH 5.5. In addition, increasing oxygen partial pressure, therefore increasing oxy-Hc fraction, resulted in larger product formation at pH 5.5. These results suggest that the reaction consists in the protonation of peroxide bound to oxy-Hc, mediated by weak acids like HF or HN_3, to give an intermediate containing a di-Cu(II)-hydroperoxo complex. This further reacts with water to give a bis-hydroxo-Cu(II) site. The proposed structure for the reaction product accounts for : i, the antiferromagnetic coupling of the two Cu(II) ions; ii, the observation that oxy-Hc and met-Hc have the same coordination number; iii, the presence of typical hydroxide-Cu(II) LMCT transition. Met-Hc reacts with azide to give a complex characterised by N_3^--Cu(II) LMCT transitions at 390-450 nm. Ligand titration experiments and spectroscopic characterisation of the complex at pH 7.0 or 5.5 indicate that stoichiometry and geometry of coordination for azide are affected by pH.

CHEMICAL MODELING OF
COPPER MONOOXYGENASE ACTIVE SITE.

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All known results suggest that dopamine β hydroxylase (DBH, type 2 monooxygenase, benzylic hydroxylation of dopamine into noradrenaline)[1] contains two inequivalent copper atoms per active site. A Cu_A site is proposed to be at the core of a reductant site where the reductor delivers one electron at a time. At a distance greater than 4 Å, a Cu_B center involved in dioxygen fixation is responsible for the hydroxylation of dopamine. Whereas it is well established that the oxidized DBH has a $Cu_A(His)_3(H_2O)\cdots Cu_B(His)_2X(H_2O)$ type configuration, the structure of the reduced DBH is not really clear. EXAFS data show the loss of 2 H_2O molecules with the appearance of 0.5 sulfur ligand per Cu(I) center in the second row scattered. While this S ligand assigned to a Met residue was first proposed to be co-ordinated to the Cu_A center, a recent preparation of half-apo reduced DBH seems to demonstrate that the MetS ligand was co-ordinated to the Cu_B center.[2] The MetS ligand could be present in the oxidized form as a weakly bound axial ligand not detected by EXAFS. This situation has helped to focus attention on the chemistry and the X-ray spectroscopy of copper ions with N_3S type ligand. We report here the synthesis and the characterization of Cu(I) and Cu(II) complexes co-ordinated to $MeS(CH_2)_2PY2$ ligand by X-ray spectroscopy and their reactivity towards dioxygen and oxygen donnors.

(1)   Stewart L. C..; Klinman, J. P. *Ann. Rev. Biochem.*, **1988**, *57*, 551-92.
(2)   Reedy, B. J.; Blackburn, N. J. *J. Am. Chem. Soc.* **1994**, *116*, 1924-1931.

X-Ray absorption fine structure of Gd(III) complexes used in magnetic resonance imaging

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Chelate complexes of gadolinium have been widely examined as they are efficient relaxation agents for the water proton spins in aqueous systems : the most widely used complexes are Gd(DTPA)$^{2-}$ and Gd(DOTA)$^{-}$.

Structural informations are known and obtained on the crystalline form of these complexes by X-ray diffraction. For instance concerning Gd(DOTA)$^{-}$, the coordination sphere of the gadolinium is formed by eight-coordinated ligands and one H_2O molecule. However no structural data are available about these complexes in aqueous solution and many properties presented by these complexes remain to-day without any interpretation.

We are interested in revealing eventual changes of the structures when passing from crystalline state to the solution form, or when changing ligands or rare earth natures in correlation with the variability observed in the relaxation properties of the complexes.

The XAFS spectroscopy is a well suited technique in order to study gadolinium local structures despite the existence or absence of ordering in the system (crystalline and solution forms) . The modern theory of XAFS is based on the formalism of multiple scattering and it presents a possibility of obtaining detailed information about short and medium range order (nearly up to 5Å). We performed XAFS experiments using high intensity synchrotron radiation at LURE Laboratory (Beamline EXAFS2 - DCI - Orsay -France) on Gd(DTPA)$^{2-}$ and Gd(DOTA)$^{-}$ complexes by registering the LIII edge spectra. These complexes have been studied in crystalline forms, or in aqueous solutions presenting various dilutions and different pH. Moreover, also interested in the interaction of these contrast agents with protein; we have followed the evolution of the gadolinium coordination sphere (aqueous solution pH 7.4) by addition with 5% or 2% serum-albumine solution.

In each of these cases we observed light but significant changes in the Fourier transforms of the spectra from 2 to 4 A where single scattering of the first coordination sphere and single and multiple scattering effects in donor or soft bounded ligands are expected. We thus evidence the sensitivity of XAFS technique to approach such a problem.

References

-Dian Chen, P.J.Squattrito, A.E. Martell, A.Clearfield
    Inorg.Chem.1990, 29, 4366-4368.
-M.S.Konings, W.C.Dow, D.B.Love, K.N.Raymond, S.C.Quay, S.M.Rocklage
    Inorg.Chem 1990, 29, 1488-1491.
-F.Chaubet, M.Nguyen-Van-Duong, J.Courtieu, A.Gaudemer, A.Gred, A.L.Crumbliss
    Can.J.Chem, 1991, 69, 1107.

Assessment of Structural Determination by X-Ray Absorption Spectroscopy with Polyimidazole Copper Complexes and Polyimidazole Thioether Copper Complexes.

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A number of metalloenzymes contain copper in their active site. Among these the coordination sphere of the copper consists often of imidazole (His), thiolate (Cys), and thioether (Met) ligands. Some of these copper enzymes are well characterized, such as azurin and plastocyanin. Their structures have been determined by X-ray diffraction. An other structural tool is X-ray absorption spectroscopy (XAFS) which gives detailed information about the local structure around the x-ray absorbing atom (for instance a Cu atom),[1] independent of the ordering in the sytem (crystalline state or solution).
A series of copper complexes has been prepared within a model study of copper proteins.[2] Among these, complexes containing two to four imidazole ligands (see complex **1**, four imidazole ligands), and zero to two thioether ligands (see complex **2**, two imidazole and two thioether ligands) have been selected for a systematic XAFS study. The structures of all these complexes have been determined by X-ray diffraction.

1   **2**
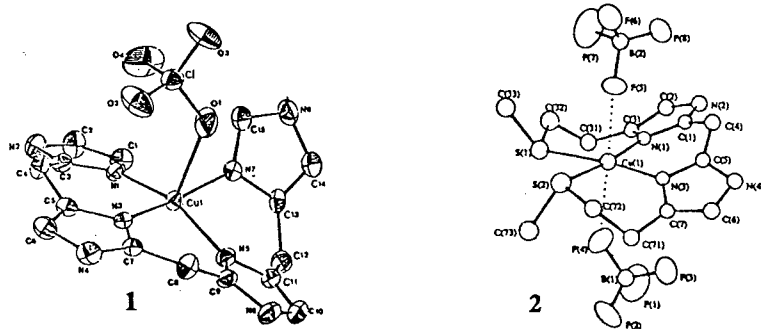
We did Cu Kedge measurements (DCI-Lure-Orsay-France) of these complexes and noted within this series an evolution of the radial distribution function obtained by Fourier transform of the spectra. For the assignments of the observed peaks we have used the latest theory of XAFS (FEFF6 program).[3] This is based on the formalism of single (SS) and multilple (MS) scattering of the photoelectron emitted during the absorption process. As has been shown previously, very intense multiple scattering signals are present in the XAFS spectra of the complexes studied which are related to the existence of the well defined structure of the imidazole ring.
For the complexes studied we have done MS calculations and we have analyzed the full XAFS signals and their radial distribution functions. The analysis has shown that in order to simulate the experimental data it is necessary to take into account SS and MS paths in the imidazole ring from 1.9 to about 5 Å which have led to a good agreement with the x-ray diffraction data. The first peak is attributed to SS processes in the first shells composed of Cu-N bonding with the imidazole rings. The next peaks are due to SS and MS processes which reflect the type of ligand.

1   EXAFS Studies of Copper Proteins- N.J.Blackburn- *Synchrotron Radiation in Biophysics* - S.S.Hasnain - Daresbury Laboratory - U.K

2   K.C.Tran, J. P. Battioni, J. L. Zimmermann, C. Bois, G.J.A.A. Koolhaas, P. Leduc, E. Mulliez, H. Boumchita, J. Reedijk, J.C. Chottard, *Inorg. Chem.* **1994**, in press.

3   J.J. Rehr, J. Muster de Leon, S. I. Zabinsky, R. C. Albers, *J. Am. Chem. Soc.* **1991**, *113*, 5135.

^{1}H NMR studies of the CoFe$_3$S$_4$ derivative of ferredoxin from *Clostridium acidi urici*

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Clostridium acidi urici ferredoxin (Fd) is a small iron-sulfur protein (MW 6,000), containing two Fe$_4$S$_4$ clusters. The conversion of the Fe$_4$S$_4$ clusters into the corresponding Fe$_3$S$_4$ species is known.[1] The possibility of the reconstitution of the Fe$_4$S$_4$ stucture by uptake of an iron site with others metal ions opened new chemical pathways for the synthesis of the mixed metal structures of the type MFe$_3$S$_4$.[2]

The iron ion is removed only from cluster I by treatment of the *Clostridium acidi urici* Fd with 3-fold molar excess of ferricyanide and metals such as Fe, Zn and Co can be inserted by incubation with the correspondent divalent metal ion under reducing condition to give MFe$_3$S$_4$ clusters.[3]

We undertook ^{1}H NMR studies of oxidized and reduced [CoFe$_3$S$_4$] derivative. The spectrum of the reduced species shows a set of narrow signals whose number exceeds what expected for a single species (Fig.1A). With time, the formation of another [CoFe$_3$S$_4$]$^{1+}$ species was observed (Fig.1B), in slow exchange with the first one. The conversion was complete in a few hours (Fig.1C).
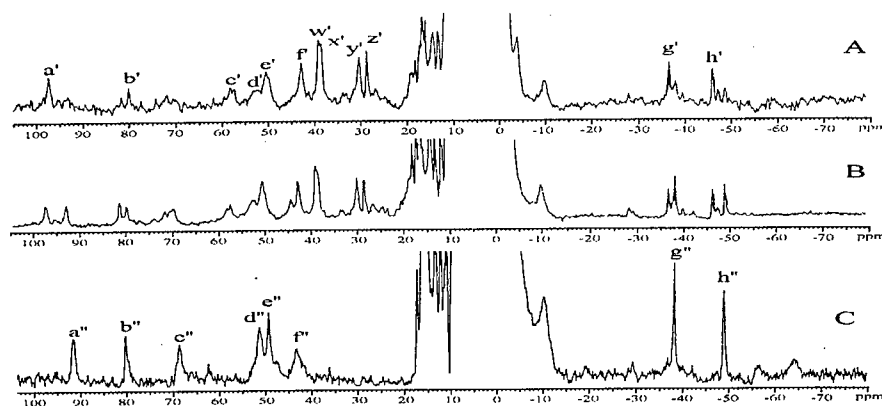

Fig.1 - 300 MHz, ^{1}H NMR spectra of the [CoFe$_3$S$_4$]$^{1+}$ derivative of *C. acidi urici* ferredoxin. A, 291 K; B and C, 293K

The pattern of the shifts and the temperature dependence can be reproduced with an Hamiltonian of the type

$$\hat{\mathcal{H}} = J(\hat{S}_1 \cdot \hat{S}_2 + \hat{S}_1 \cdot \hat{S}_3 + \hat{S}_1 \cdot \hat{S}_4 + \hat{S}_2 \cdot \hat{S}_3 + \hat{S}_2 \cdot \hat{S}_4 + \hat{S}_3 \cdot \hat{S}_4) + \Delta J_{12}\hat{S}_1 \cdot \hat{S}_2 + \Delta J_{123}(\hat{S}_1 \cdot \hat{S}_2 + \hat{S}_1 \cdot \hat{S}_3 + \hat{S}_2 \cdot \hat{S}_3),$$

where S$_1$, S$_2$ refer to the mixed valence pair Fe$^{2.5+}$, S$_3$ refers to Fe^{2+} ion and S^4 to the Co^{2+} ion.[3]

The exchange coupling costants seem to be larger between Co and Fe than within the Fe ions (J$_{Co, Fe}$=500 cm^{-1}, J$_{Fe^{2.5+}, Fe^{2+}}$= 250 cm^{-1}, J$_{Fe^{2.5+}, Fe^{2.5+}}$= 248 cm^{-1}).

1. Bertini, I.; Briganti, F.; Calzolai, L.; Messori, L. and Scozzafava, A. *FEBS Lett.* 332, 268 (1993)
2. Moura, J. J. G.; Macedo, A. L.; Moreno, C. and Moura, J. *NATO ASI Ser., Ser. C* 371 (Supramol. Chem.), 403 (1992)..
3. Benelli, B.; Bertini, I.; Capozzi, F. and Luchinat, C. *submitted* (1994).

Surface plasmon resonance as a new method for detection of any changes in mioglobin dielectric properties

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It is known [1], that surface plasmon resonance (SPR) can be applied for detection of the thicknes and dielectric constant of thin dielectric layers. We used this method for determination of a changes in dielectric constant of mioglobin under internal and external influence. Dielectric properties of mioglobin have been studed on pH, nature of solvent, temperature (the internal factors) and electromagnetic field action (the extexnal factor).

We measured the angle of the minimum reflection coefficient in the system Ag layer-mioglobin in the process of adsorbtion. For the experiments we used the setup described below. The HeNe lazer beam (wavelength 0.63 mcm) was directed to the total reflection prizm. One side of prizm which was covered by Ag layer with thiknees about 0.5 mcm simultaneously was a wall of the flow cell. The flow cell with mioglobin solution is placed on a rotation table of the device gs-5. In experiment an accuracy of the detection of SPR angle in Ag layer on quartz prizm was 5". We investigated the mioglobin solution (0.1-0.001%) in phosphate bufer pH 7-9. The cuvetsystem was irradiated by millimeter wave from generator g4-141. Density of the input power was 50-60 mW/sq.cm , frequency range - 37-53 GHz.

Changes of solvent from water to phosphate bufer causes to drastic SPR angle change in about several degrees. Changes of pH and temperature (14-26 degree) couses to angles changes from several minutes to several seconds. Millimeter radiation changes the biomolecule adsorption value. Swiching on does not influence on the SPR angle. However long irradiation (during 10-15 min) of the cuvetsystem with mioglobin solution under satureted adsorption causes to futher changes of the adsorbtion and SPR angle changes.

On the base of the experiment we accounted dielectric constant of mioglobin and its changes.

1.Helene E.de Bruijn, Aufried T.M.Lenferink, Rob P.H.Kooyman and Jan Greve, Optics Communications (1991) 86, 444-448.

REACTIONS OF *trans*-(NH$_3$)$_2$Pt(II)-COMPOUNDS WITH SHORT OLIGONUCLEOTIDES

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Among the approaches of selectively targetting nucleic acids, the concept of antisense or antigene oligonucleotides is particulary attractive. There have been many attempts to increase the binding affinity of an oligonucleotide for a given DNA or RNA sequence, eg. by adding intercalating agents to the oligonucleotide, by use of artificial bases or addition of photo-crosslinking entities to the oligonucleotides, etc.[1-4].

Our approach has been to use kinetically inert metal ions (eg. Pt(II)), to form an irreversible crosslink with an oligonucleotide and a target sequence.

As starting materials for the platination we chose the tetranucleotides 5´d(TTTC)3´ and 5´d(CTTT)3´. We achieved monofunctional platination at C-N3-position in both cases with *trans*-[(NH$_3$)$_2$Pt(1-MeT)Cl] (1-MeT = 1-methylthymine anion). Our attempts now focus on the replacement of 1-MeT by 5´d(TGAAAT)3´. Preparative and NMR spectroscopic studies will be presented.

References

(1) Uhlmann, E.; Peyman, A.; Chem. Rev. 90 (1990), 543-584

(2) Miller et al. "Molecular Mechanisms of Carcinogenic and Antitumor Activity", Chagas, C.; Pullman, B. (Editors), Pontif. Acad. Scient. Scripta Varia, Adenin Press (1987), 169-203

(3) Hélène, C.; Thuong, N.T.; "Molecular Mechanisms of Carcinogenic and Antitumor Activity", Chagas, C.; Pullman, B. (Editors), Pontif. Acad. Scient. Scripta Varia, Adenin Press (1987), 205-222

(4) Thuong, N.T.; Hélène, C.; Angew. Chem. 105 (1993), 697-723

NICKEL(II) AND COPPER(II) COMPLEXES OF BnMA. X-RAY POWDER DIFFRACTION AND KINETIC STUDY OF THE DECOMPOSITION PROCESSES.

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As a continuation of our researchs [1,2] on metal-carbohydrate α-amino acid complexes, we report here the results obtained from X-ray powder diffraction and kinetic thermal decomposition studies of $Ni(BnMa)_2.2H_2O$ and $Cu(BnMa)_2.2H_2O$ (BnMa = 2-benzylamino-2-deoxy-D-glycero-D-talo heptonic acid).

The X-ray powder patterns show that both compounds cristallize in the monoclinic system. The systematic extinctions (0k0) absent for k = odd and (h0l) for l = odd indicate that the most probable space group is $P2_1/c$. The refined parameters of the unit cell are: a = 12.534(5) Å; b = 22.958(7) Å; c = 11.082(4) Å; b = 89.78(4)°; V = 3189(1) Å^3, for $Ni(BnMa)_2.2H_2O$ and a = 12.522(4) Å; b =23.392(6) Å; c = 10.849(3) Å; b = 88.98(3)°; V = 3177(1) Å^3, for $Cu(BnMa)_2.2H_2O$. These compounds are isomorphous with the analogous Zn(II) and Cd(II) complexes [3].

The dehydration and the decomposition processes of both complexes allows us to indicate that these processes can best be described as a three-dimensional diffusion-controlled reaction. The activation energy using non-isothermal methods were calculated.

1. M.A. Díaz Díez, F.J. García Barros, E. Sabio Rey and C. Valenzuela Calahorro, *J.Inorg.Biochem.*, **48**, 129 (1992).
2. M.A. Díaz Díez, F.J. García Barros, E. Sabio Rey and C. Valenzuela Calahorro, *J.Inorg.Biochem.*, **53**, 109 (1994).
3. M.A. Díaz Díez, F.J. García Barros, F.J. Higes Rolando, A. Bernalte García and C. Valenzuela Calahorro, *Polyhedron*, **12**, 2519 (1993).

SYNTHETIC ANALOGS OF THE PURPLE ACID PHOSPHATASES :

STRUCTURAL AND MAGNETIC PROPERTIES

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The purple acid phosphatases are non heminic enzymes active in the hydrolysis of phosphates. Extensive spectroscopic studies have led to propose that the two iron atoms are bridged by an oxygen ligand (O, OH) and a carboxylate. A distinctive feature of these enzymes is the coordination of the diiron unit by a single terminal tyrosine. The enzyme exists in an inactive oxidized Fe^{III} Fe^{III} form and a reduced Fe^{II} Fe^{III} active one. Surprisingly no diferrous state exists since ferrous ions are released on dithionite reduction.

We will describe the structure and the magnetic properties of the first synthetic analogs of the PAP enzyme which reproduce the unsymmetric binding of a phenolate to a diiron unit (Figure 1). In addition, electrochemical studies bring some clues on the unstability of the diferrous form of the enzymes.

Figure 1
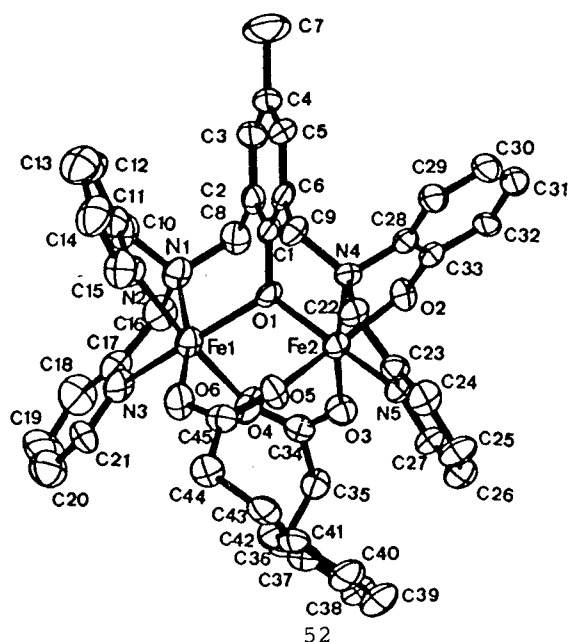
Mechanism of Electron Transfer Between Plastocyanin and Optically Active Complexes Based on Stereoselectivity Measurements with Native and Mutant Recombinant Proteins.

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The kinetics of the electron·transfer reaction between spinach plastocyanin and various neutral optically active complexes of the types (I) and (II) have been measured as a function of the reaction temperature. The reactions are found to be stereoselective and the comparison of the activation parameters $\Delta H^{\#}$ and $\Delta S^{\#}$ suggests different pathways for the

Type I   Type II

$$X = H_2O$$

$$M = Fe^{II} ; Co^{II}$$
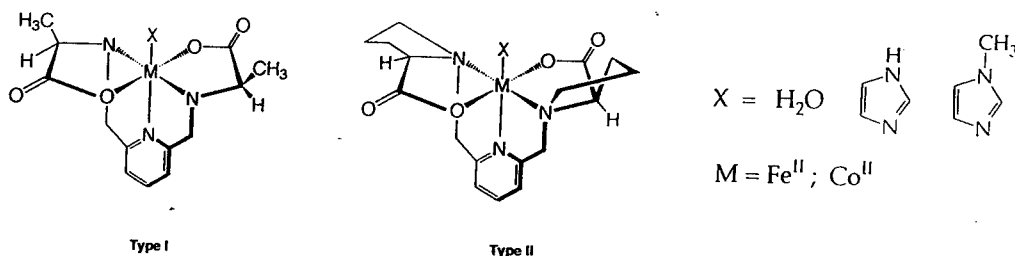
electron transfer at the reactive site located near the Cu^{2+} atom (adjacent site). With Co^{2+} as the reductant the electron transfer is strongly catalyzed by the presence of imidazole and N-methyl-imidazole. An edge-to-edge transfer from the imidazole on the Co^{2+} to the histidine(87) coordinated to the Cu^{2+} atom would explain this catalysis (Figure)

The kinetics of Cu^{2+} sequestration from plastocyanin has been determined and is discussed as a function of the size of the ligands used.

In order to verify the proposed mechanism the stereoselectivity of the electron transfer and the kinetics of Cu^{2+} sequestration is compared with the corresponding data obtained for site-directed mutants of the recombinant protein.
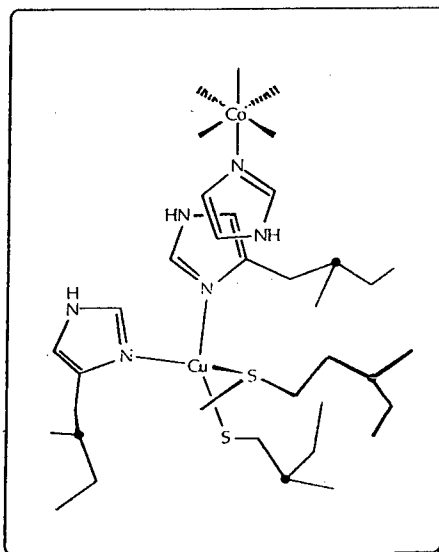
XAS Studies on the Catechol 2,3 Dioxygenase from P. putida MT2

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Dioxygenases play a primary role in the biodegradation of most aromatic compounds in the biosphere. In particular, catechol 2,3 dioxygenase (C2,3O) from Pseudomonas putida MT2 which contains one iron(II) ion for each of the four identical 32,000 MW subunits catalyzes the extradiol cleavage of the aromatic ring of catechols. Very little is known on the structure-function relationship of such enzymes due to their instability and to the difficulties encountered in the spectroscopic study of high spin iron(II) centers.

For such a reason we performed XAS (X-ray Absorption Spectroscopy) measurements on the C2,3O enzyme. The pre-edge (1s-3d transition) peak analysis of the XAS spectrum of native C2,3O is indicative of the presence of an hexa-coordinated iron(II) ion into its active site. These data are also supported by the analysis of the EXAFS spectrum which shows that the first coordination sphere of the iron ion is most probably formed by four oxygen and two nitrogen atoms at 2.02 Å and 2.17 Å respectively. The characteristic features of histidine residues bound to the iron are visible in the EXAFS spectrum and its Fourier Transform. A quantitative analysis suggests the presence of two imidazole rings bound to the active site iron(II).

The binding of inhibitors (2Cl- and 2Br-phenols), substate analogues (2OH-pyridine-N-oxide) and substrates (catechol) cause significant variations to the XAS spectra indicating that all of them bind to the active site iron. In all these cases the coordination number (six) and the geometry remain essentially unchanged with respect to the native enzyme. This implies that water molecules or proteic oxygen ligands are displaced by the inhibitor/substrate molecules because we observed that the histidine ligands always remain coordinated to the metal ion.

A model containing bidentate complexes of the catechol and 2-hydroxy-pyridine-N-oxide adducts to the active site iron ion is consistent with the experimental data whereas a monodentate model was able to give satisfactory results only for the 2Cl- and 2Br-phenols inhibitors.

Our data appear to be in agreement with the Mössbauer data indicating a substitutional variability at the metal center without affecting the coordination number and geometry.

A   B   C   D
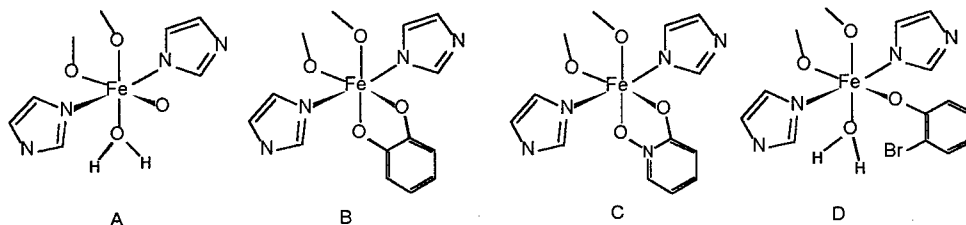
The scheme shows the active site of native C2,3O (A), and its complexes with catechol (B), 2hydroxy-pyridine-N-Oxide (C) and 2-bromo-phenol (D) as resulting from the analysis of our data.

A MONOMERIC ANALOG OF CU,ZN SUPEROXIDE DISMUTASE INVESTIGATED THROUGH SPECTROSCOPIC TECHNIQUES

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In copper,zinc superoxide dismutase (SOD) the two subunits are linked together by hydrophobic interactions. We have substituted, through site specific mutagenesis, two residues which are responsible of these interactions, Phe 50 and Gly 51, by two Glu's. This kind of modification produces a stable monomeric analog of SOD.

The mutated protein, characterized by electronic spectroscopies, EPR, NMRD and ^{1}H NMR techniques, shows some modification in the arrangement of the ligands at the copper site. Increases in the axiality of the copper chromophore and the $Cu-OH_2$ distance have been observed. These structural changes have been related to a remodelling of the newly exposed interface which would probably indirectly affect the active site.

Furthermore, NMR signals linewidth of both reduced copper(I),zinc(II) SOD and copper(II),cobalt(II) derivative are decreased of about a factor of two as a consequence of the lower molecular weight of the protein. This enhances resolution and affords additional structural information.

The affinity for the anion N_3^- and the activity of the monomeric enzyme towards superoxide have been also tested. The observed decrease in these two parameters have been correlated to the introduction of two negative charges on the surface and/or to the restructuring of the exposed dimeric interface with concomitant shifts of charged residues which could modify electrostatic forces during substrate docking.

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GOLD(III) MODIFICATION OF TRI- AND TETRAPEPTIDES: SIDE-EFFECTS OF CHRYSOTHERAPY

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Gold(III) generated via myeloperoxidase is thought to play an important role in the side-effects of anti-arthritic gold drugs [1, 2]. Au(III) modified peptides bound to MHC class II molecules may be involved in delayed hypersensitivity reactions ('gold rashes') observed in patients undergoing chrysotherapy, but little is known about the binding of Au(III) to peptides.

We present studies of the interaction of Au(III) with the tripeptide Gly-Gly-His (GGH) and tetrapeptide Val-Ile-His-Asn (VIHN). Reaction of GGH with Au(III) at low pH (1.5-2) is slow ($T_{1/2}$ ca. 6h, 10 mM, 310 K) and involves one intermediate. The final product is shown by X-ray crystallography to be the square-planar complex [Au(III) (H_{-2}-Gly-Gly-L-His)]Cl.H_2O containing Au(III) coordinated to the terminal NH_2, two deprotonated peptide NH's and His Nδ.

The course of reaction of Au(III) with VIHN is more complicated and several products have been characterized by NMR spectroscopy.

[1] B. Beverly and D. Couri, Fed. Proc. 46: 854 (1987)

[2] D. Schuhmann, M. Kubicka-Muranyi, J. Mirtschewa, J. Günther, P. Kind, and E. Gleichmann, J. Immunol. 145: 2132-2139 (1990)

Copper coordination of poly-imidazole ligands

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In the course of our investigations on copper coordination compounds as potential analogues of the active sites of copper proteins, a series of poly-imidazole alcohol ligands have been designed and synthesised.
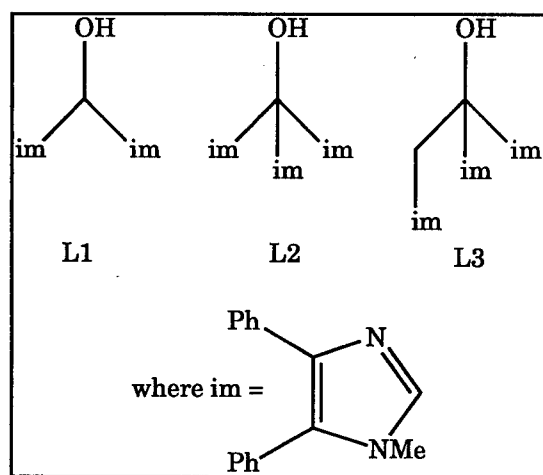
Our approach has involved the development of synthetic procedures to prepare novel imidazole ligands. In particular, the introduction of steric constraints within the imidazole ligand has resulted in the formation of copper complexes with low coordination numbers.

Also, the structural characterisation of these systems has revealed that, in addition to the imidazoles binding to the copper centre, the hydroxy-group of these ligands can be deprotonated and can also bind to the copper centre.

These studies have resulted in the synthesis of a series of monomeric, dimeric, trimeric and tetrameric copper systems. The structure and properties of a selection of these complexes will be presented.

NOVEL MACROCYCLIC DINUCLEAR COPPER (II) COMPLEXES AS MODELSYSTEMS FOR TYPE II AND III COPPER SITES.

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There is considerable interest in dinuclear copper complexes as model systems for the active site of copper enzymes like Dopamine-β-hydroxylase (type II) or Tyrosinase (type III). Dinuclear copper complexes with macrocyclic ligands have demonstrated to catalyse the oxidation of various substrates like catechols or phenols. Several di-copper (and di-cobalt) complexes are also capable of binding dioxygen reversibly and are therefore important as functional models for metallo-enzymes involved in O_2 activation.

Based on the original synthetic approach of Tarrago *et al.*[1], using a modified procedure, we obtained a new octadentate macrocyclic ligand containing four pyrazole sp^2 donor groups of low basicity and four amine sp^3 nitrogen donor groups. The 30-membered macrocycle appears to be capable of coordinating two metal ions at approximately 7 Å distance. Dinuclear complexes containing Cu(II) or Co(II) ions with non-coordinating anions have been obtained. An X-ray structure determination of a dinuclear Cu(II) complex, including a chloride anion, confirmed the (2+2) ring size of the macrocyclic ligand.
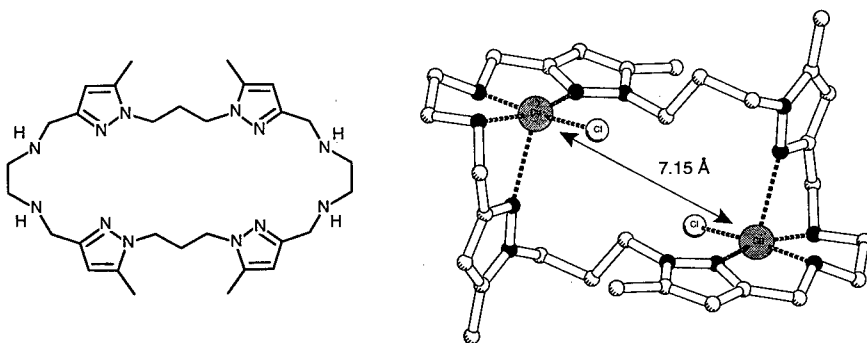
Preliminary results show that the new macrocyclic ligand is capable of coordinating Cu ions in both the Cu(I) and Cu(II) state. Efforts are undertaken to insert bridging anions between the copper centres. Reactivity studies to explore the (catalytic) oxidation of various substrates in combination with OH⁻ and H_2O_2 will be presented.

1.   G. Tarrago, S. El-Kadiri, C. Coquelet, *New J. Chem.*, **15**, 677 (1991).

REFINED CRYSTAL STRUCTURES OF NITROGENASE MOFE PROTEINS AT 2.2 Å RESOLUTION: THE METAL CLUSTERS AND THEIR ENVIRONMENTS.

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Mo-dependent nitrogenases comprise two separately purifiable metalloproteins called MoFe protein and Fe protein. MoFe-protein, which contains the site of substrate reduction, is an $\alpha_2\beta_2$ tetramer ($M_r \cong$ 220-230kDa) and binds 2 Mo and 30 Fe atoms in the form of two unusual types of metal-sulfur clusters known as the FeMo-cofactor and P-clusters.

We have determined and refined crystal structures of the MoFe protein from *Clostridium pasteurianum* (Cp1) [2] and *Azotobacter vinelandii* using diffraction data of 2.2 Å resolution. The R-factor for the refined model of Cp1 is 15%, based on all measured data, whereas that for Av1 is 18%. Selected features of these structures will be presented.

The stereochemistry of the metal-sulfur clusters as well as their interactions with protein groups and bound water molecules will be considered in detail. Throughout the analysis we have made use of anomalous diffraction methods specifically to probe and define the structures of the metal-sulfur groups. The results of refinement experiments designed to test the reliability of different models for the structures of the clusters also will be reported, as will comparisons to the models published by Rees and coworkers [3].

2. J.T. Bolin *et al.* (1993) in *Molybdenum Enzymes, Cofactors and Models*, E. Stiefel *et al.* (Eds.), American Chemical Society, Washington, D.C., pp. 186-195.

3. J. Kim and D.C. Rees, *Science*, **257**, 1677-1682 (1992); M.K. Chan, J. Kim and D.C. Rees, *Science*, **260**, 792-794 (1993).

THE X-RAY CRYSTAL STRUCTURE OF 2,3-DIHYDROXYBIPHENYL 1,2-DIOXYGENASE, A NON-HEME, FE(II)-ENZYME THAT CATALYZES EXTRADIOL CLEAVAGE OF AROMATIC RINGS.

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Extradiol dioxygenases are a class of non-heme Fe(II)-dependent enzymes that play critical roles in the catabolism of aromatic compounds by virtue of their ability to catalyze the cleavage of aromatic rings. We have used X-ray crystallographic techniques to determine, under anaerobic conditions, the structure of an extradiol dioxygenase involved in the degradation of biphenyl and polychlorinated biphenyls (PCBs), the 2,3-dihydroxybiphenyl 1,2-dioxygenase from *Pseudomonas* sp. strain LB400.

The structure of the enzyme will be described and related to its function as well as to the structures and functions of similar enzymes. In terms of the polypeptide fold, the structure shows two levels of internal repetition as well as topological features that appear to be different from other known protein structures. The Fe- and substrate–binding sites are located within a cylindrical cavity that is topologically different from previously observed barrel-like structures. In terms of this location and the details of the Fe binding site, the structure is different from the known structures of other non-heme Fe-dependent dioxygenases such as protocatechuate 3,4-dioxygenase and soybean lipoxygenase L1.

Histidyl-Glycyl containing Peptides: A Spectroscopic Characterization of Copper(II) Interaction with the linear octapeptide H-(L-His-Gly)₄-OH.

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The design of new peptide models and the study of their interaction with metal ions involve a considerable interest. The biomimetic approach allows to understand the factors that control the molecular organisation, the metal induced conformations for ion-transport processes and the metal ion binding mode at the active site of metalloproteins. The histidyl-containing peptides are suitable models since histidyl residues play an important role in a large number of enzymatic processes. Transition metal ions can be used as peptide side-chain cross linking agents. In particular the Cu(II) ion is often involved, at the physiological pH, in the co-ordination of the active site of metalloproteins through the imidazole ring of the histidine residues.

We performed a large scale synthesis of the tetrapeptide and of the linear and cyclic octapeptide containing alternated histidyl and glycyl residues both in solution and combining the solid and liquid phase techniques [1-3]. The interaction of the tetrapeptide H-(L-His-Gly)₂-OMe with Cu(II), VO(IV) and Mn(II) have been investigated [4] at different pH and metal-ligand molar ratios by a spectroscopic approach in order to clarify the specificity of the metal-ligand interaction. The study of Cu(II)-cyclo(L-His-Gly)₄ has shown [5] that this peptide can be a right model system in physiological conditions when the metal binding mode is imidazole-mediated.

We report here the results for the interaction of the linear octapeptide H-(L-His-Gly)₄-OH with Cu(II) ion at different pH and metal-ligand molar ratios starting from an acidic equimolar solution. The nature and structure of copper(II) complexes of this multidentate and flexible ligand have been assessed by different spectroscopic techniques (UV, EPR and NMR). The UV/VIS position of λ_{max}^{d-d} characteristic of equatorial nitrogen or oxygen co-ordination indicates the presence of at least three different species. The computer calculated EPR spectra (room temperature and 77 K) confirm, together with the ¹H NMR analysis, the presence of different species with increasing co-ordination of equatorial nitrogen atoms when the pH value rises.

References

1 M. Chelli, M. Ginanneschi, A.M. Papini, D. Pinzani, G. Rapi, E. Borghi, F. Laschi, M.Occhiuzzi, J. Chem. Res. (M and S) 1993, (M), 2928-2936; (S), 437.
2 M. Chelli, M. Ginanneschi, A.M. Papini, D. Pinzani, G. Rapi, in Peptides 1992, 1993, C.H. Schneider and A.N. Eberle Eds., ESCOM Science Publishers B. V., pp. 255-256.
3 M. Chelli, M. Ginanneschi, A.M. Papini, D. Pinzani, G. Rapi, J. Chem.Res. (S) 1993, 118-119.
4 E. Borghi, F. Laschi, M. Occhiuzzi, M. Chelli, M. Ginanneschi, A.M. Papini, D. Pinzani, G.Rapi, J. Chem. Res.(S) 1994, 48-49.
5 M. Chelli, M. Ginanneschi, A.M. Papini, D. Pinzani, G. Rapi, P. Rovero, E. Borghi, F. Laschi, M. Occhiuzzi, II convegno Nazionale Società Chimica Italiana, Divisione di Chimica dei Sistemi Biologici, 1993, Roma, 9-11 dicembre, p. 78.

Structural Models for Biologically Relevant Zinc-Thiolate Species

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In addition to its catalytic role zinc has an important function in determining the tertiary structure of many proteins. It does so by preferably being attached to sulfur and nitrogen donor functions of the peptide constituents, as in the zinc fingers, metallothioneines, and alcohol dehydrogenases.
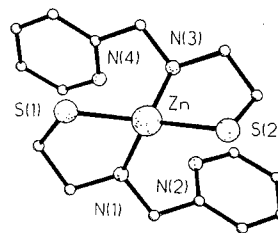
In order to model the environment of these structural zinc ions we have investigated complexes of zinc with the polydentate ligands 2-mercaptoethyl-2-picolylamine (MEPAH) and 2-mercaptophenyl-2-picolylamine (MPPAH). Structure determinations of the complexes have revealed a quite varied coordination behaviour of these ligands which can, in part, be related to the N and S coordination of zinc in the proteins.

We have observed bridging thiolates, such as those found in metallothioneines, in the bi- and trinuclear complexes $(MEPA)_2Zn_2(OAc)_2$ and $(MEPA)_4Zn_3X_2$ (with $X = BF_4$, NO_3, ClO_4). Like in the proteins it is possible to increase the coordination number of zinc by the formation of thiolate bridges.

The complexes $(MPPA)_2Zn$ and $(MEPA)_2Zn$ can be considered as model complexes for zinc fingers. They exhibit a distorted N_2S_2 coordination. The preference for this type of coordination is evident from the fact that they contain non-coordinating nitrogen donor functions.
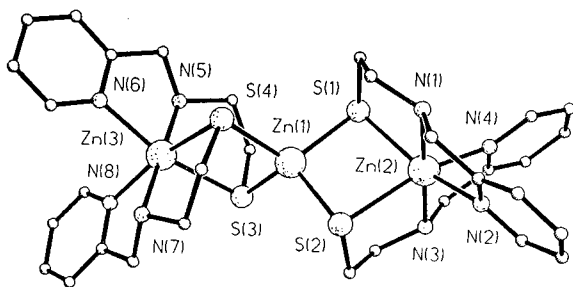
Structure of the cation $[(MEPA)_4Zn_3)]^{2+}$ Structure of $(MEPA)_2Zn$

This excess of donor functions in $(MEPA)_2Zn$ seems to be the reason for an unusual reaction of the complex with methylene chloride during which Cl is coordinated to zinc with formation of $[(MEPA)ZnCl]_n$ while CH_2Cl seems to be attached to the sulfur function of a MEPA ligand. The relation of this reaction to the function of a E. coli DNA repair domain will be discussed.

NMR Characterization of Ala80 Cytochrome *c*

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The substitution of the axial methionine ligand with alanine in *S. cerevisiae* iso-1-cytochrome *c* produces a mutant (Ala80cyt *c*) which has coordination properties similar to globins and peroxidases. The pH dependence of the ^{1}H NMR spectrum of ferri-Ala80cyt *c* confirms that the heme iron undergoes a change in coordination concomitant with a change in spin state with a pK_a around 6, which correlates well with the value of 6.2 obtained from absorption spectroscopy.[1,2] The NMR spectra in the pH range 2.2-11 show the existence of many equilibria which affect the NMR parameters of the hyperfine-shifted resonances, indicating that the equilibria involve the protonation of residues close to the active site. Comparison of the spectral behavior with that of wild type cyt *c* aids in the interpretation of the spectra of wild type at low and high pH values. Due to the presence of multiple forms of the mutant at any pH value, the CN⁻ adduct of Ala80cyt *c* has been studied for the purpose of obtaining more information on the solution structure of the mutant. Using ^{1}H NOESY, COSY, and TOCSY experiments, assignments have been made for the heme substituents as well as the axial histidine. Using this as a starting point, we have been able to extend the assignment to residues that experience some paramagnetic effect as well as residues which are farther from the heme iron.

1) Bren, K.L.; Gray, H.B. *J. Am.Chem. Soc.* **1993**, *115*,10382-10383.
2) Lu, Y.; Casimiro, D.R.; Bren, K.L; Richards, J.H.; Gray, H.B. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11456-11459.

Spectroscopic characterisation of a novel high-potential Copper-Iron-Sulphur cluster in a ferredoxin.

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The heterometal cluster $[Cu:3Fe-4S]^{2+/1+}$ has been formed in ferredoxin III from *D.africanus* (DaIII) and characterised spectroscopically in both oxidation states. MCD spectra of the copper adduct were recorded and showed a dramatic change in shape compared to the spectra of the $[3Fe-4S]^{1+/0}$ cluster, although the spin ground state for both oxidation states was found unchanged. EPR and MCD spectroscopies thus support the assignment as $[Cu:3Fe-4S]^{2+}$ ($S=1/2$) and $[Cu:3Fe-4S]^{1+}$ ($S=2$), with Cu bound formally as Cu(I) [1].

The accompanying electrochemical investigation showed that the binding was reversible and that cyanide could cause the selective loss of Cu and the regeneration of the [3Fe-4S] reactant. It also enabled the determination of the high midpoint redox potential of this cluster and the binding affinity for Cu. By comparison with earlier work [2,3], the affinity order of the reduced 3Fe cluster for M^{n+} was found to be :

$$Cu(I) > > Tl(I) \approx Cd(II) > Zn(II) > Fe(II).$$
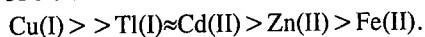
References :

[1] Butt J.N., Niles J., Armstrong F.A., Breton J., Thomson A.J., in press.

[2] Butt J.N., Armstrong F.A., Breton J., Georges S.J., Thomson A.J., Hatchikian E.C., (1991), J.Amer.Chem.Soc., **113**, 6663-6670.

[3] Butt J.N., Sucheta A., Armstrong F.A., Breton J., Thomson A.J., Hatchikian E.C., (1991), J.Amer.Chem.Soc., **113**, 8948-8950.

THE CYTOCHROME C$_3$ SUPERFAMILY :
STRUCTURAL RELATIONSHIPS AMONG TETRAHEMIC CYTOCHROME C$_3$, CYTOCHROME C$_3$ (Mr 26000) AND (16 HEME) HIGH MOLECULAR WEIGHT CYTOCHROME C FROM DESULFOVIBRIO

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Sulfate reducing bacteria derive energy for metabolic processes from sulfate respiration. Some striking differences from other respiratory modes are the negative redox potential involved and the presence of multihemic cytochromes c with no structural similarity to other cytochrome c classes. Desulfovibrio contains three different multihemic c - type cytochromes : the tetraheme cytochrome c$_3$ (Mr 13000), the octaheme cytochrome c$_3$ (Mr 26000), and a high molecular weight cytochrome c (Hmc) with 16 hemes and a (65500 Da) polypeptide chain. The four hemes of cytochrome c$_3$ exhibit a distinct redox potential in the -200 to -400 mV range and all have the same His-His iron atom axial ligands. The D. d. Norway cytochrome c$_3$ (Mr 26000) comprises two identical subunites of 111 aminoacids with the characteristics typical of tetrahemic cytochrome c$_3$ class and might be considered as a homodimeric cytochrome c$_3$. However, comparisons between these aminoacid sequences and physicodogical properties of cytochromes showed the existence of considerable differences. A high molecular weight cytochrome c has been characterized in *Desulfovibrio vulgaris*. EPR and electrochemical studies gave evidence for the presence of 16 hemes in the molecule.

A comparison on the arrangement of the heme binding sites and coordinated histidines in the amino acid sequences of cytochromes c$_3$ and Hmc has shown that the sixteen hemes are distributed in four domains, three of which are complete cytochrome c$_3$-like domains, while the fourth is an incomplete cytochrome c$_3$-like domain. The cloning of the *hmc* gene has demonstrated that this cytochrome is periplasmic. One important finding is that Hmc appears to be a component of a transmembrane electron gate to the cytoplasm. The *hmc* gene is the first open reading frame of an operon which was found to comprise several reading frames encoding membrane electron transfer proteins.

The role of cytochrome c$_3$-like domains in the folding of the polyhemic cytochromes c from *Desulfovibrio* needs to be interpreted in terms of affinity of these cytochromes with the membrane and specific interactions between these cytochromes and their redox partners.

The results of recent experiments on the interaction of the multihemic cytochromes with lipid monolayers are discussed.

The resolution of the three-dimensional structure of D. d. Norway cytochrome c$_3$ (Mr 26000), the evaluation of the homologies within the cytochrome c$_3$ superfamily and of the gene duplication in Hmc provide structural informations on the interaction with the redox partners and on the mecanism underlying the evolution of the polyhemic cytochromes.

REFERENCES
POLLOCK W.B.R., LOUTFI M., BRUSCHI M., RAPP-GILES B. J., WALL J. D. and VOORDOUW G. (1991) J. Bacteriol. 173, 220-228
BRUSCHI M., BERTRAND P., MORE C., LEROY G., BONICEL J., HALADJIAN J., CHOTTARD G., POLLOCK W. B. R. and VOORDOUW G. (1992) Biochemistry, 31, 3281-3287
BRUSCHI M., G. LEROY, F. GUERLESQUIN, J. BONICEL Biochim. Biophys. Acta (1994) sous presse.
FLORENS L., IVANOVA M., DOLLA A., CZJZEK M., HASER R., VERGER R. and BRUSCHI M. (submitted)

Steady state kinetics of barley peroxidase. Comparison of three different lignin monomers.

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In barley grain the most abundant peroxidase is BP 1. The polypeptide is less than 50 % identical to other known peroxidases and exists in a non-glycosylated (BP 1b) and a glycosylated (BP 1a) form. The Mr is 36.000 for BP 1b and 37.000 for BP 1a and the pI is 8.5 for both [1]. Barley peroxidase is presently under detailed study by 2D-NMR, X-ray crystallography and enzyme kinetics due to its unique reaction with hydrogen peroxide. The reaction is pH dependent with a strong increase in reactivity as the pH is lowered from pH 5 to 4. Furthermore, the reaction shows saturation kinetics at room temperature [2], a feature that has only been observed at temperatures around -20°C for other wild type peroxidases. Steady state studies of BP 1 with different peroxidase substrates at pH 4, show that BP 1 has high specific activities towards ABTS (2,2'-azino-di-[3-ethyl-benzthiazolinsulphonate(6)]), coniferyl alcohol and caffeic acid, intermediate specific activities towards ferulic acid and *p*-coumaric acid, and low specific activity towards guaiacol. The reactions with coniferyl alcohol, caffeic acid and ferulic acid will be discussed.

[1] A. Johansson, S.K. Rasmussen, J.E. Harthill and K.G. Welinder (1992) Plant Mol. Biol. 18, 1151-1161.

[2] C.B. Rasmussen, M. Bakovic, K.G. Welinder and H.B. Dunford (1993) FEBS letters 321, 102-105.

STRUCTURAL CHARACTERIZATION OF A MONONUCLEAR Cu(II) IN THE ACTIVE SITE OF *Carcinous maenas* HEMOCYANIN

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The preparation of a mononuclear Cu(II) derivative of *Carcinus maenas* hemocyanin (Cu(II)-Hc) and of it nitrite complex (Cu(II)-Hc-NO$_2^-$) are described.   Several techniques including cw EPR, ESEEM (Electron Spin Echo Envelope Modulation) and x-ray absorption spectroscopy have been used in their characterization.   The fitting of EXAFS data for the first coordination shell of Cu(II)-Hc indicates the presence of three ligands at 1.99 ± 0.01Å and a fourth one at 2.26 ± 0.01Å from the copper. The same coordination number and bond lengths were obtained for the nitrite complex. Based on simulation of three pulse ESEEM spectra of Cu(II)-Hc, three equivalent imidazole nitrogens are coupled to Cu(II).   A two pulse ESEEM experiment suggests that the protons of a water ligand are displaced when nitrite is bound.   The latter result is consistent with the invariance of the coordination number and bond lengths observed by EXAFS when nitrite binds to Cu(II).   Nitrite binding also induces a decrease in the hyperfine interaction for two of the three imidazole nitrogens detected by ESEEM as well as an alteration in site symmetry as determined by cw EPR.   An analysis of x-ray absorption edge spectra suggests coordination geometry of Cu(II)-Hc and of the nitrite complex that is intermediate between square-planar and tetrahedral. These results suggest an equivalent structure for Cu(II)-Hc-NO$_2^-$ and for the Cu(II) found in green half-met hemocyanin, a partially oxidized binuclear derivative formed in the reaction of the native protein with nitrite.

EXPRESSION OF 6-PYRUVOYL TETRAHYDROPTERIN SYNTHASE AND SITE-DIRECTED MUTAGENESIS OF THE PUTATIVE NON-HEME IRON-BINDING SITE.

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6-Pyruvoyl tetrahydropterin synthase (PTPS) catalyses the second reaction in the three-step biosynthesis from GTP to tetrahydro-biopterin (BH_4). BH_4 is the cofactor of several hydroxylases involved in neurotransmitter biosynthesis and of the NO synthases. We cloned and expressed the cDNA encoding for rat and human PTPS in E.coli. The recombinant enzymes were purified to homogeneity, and showed essentially the same biochemical properties as the native enzyme.

The rat enzyme was crystallized and the X-ray structure showed a hexameric native form arranged as a sandwich of two trimers.

In a trimer, three PTPS monomers form an unusual 12-stranded antiparallel ß-barrel that encloses a highly basic pore of 6-12 Å diameter. Surprisingly, a metal binding site in the postulated substrate binding pocket of each subunit, formed by the three histidine residues (23, 48 and 50), was localized. Residues Cys_{42} and Glu_{133} and His_{89} (consisting to another subunit) are close to the metal, but not liganding it and might serve as proton donors or acceptors during catalysis. We then analyzed the metal content of recombinant rat PTPS by the FAAS technique and found a 0.7:1 mol-ratio of iron:PTPS subunit. In a next step we mutated residues 23, 42, 48, 50, 89 and 133 in order to investigate their influence on enzyme activity and metal content. Preliminary data from enzymatic assays shows a dramatically loss of activity for all PTPS mutants.

Interaction between VO(IV) and inorganic and organic phosphates

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Vanadium is an important trace element and has been established to possess biological activity in various living organisms. As a fairly hard metal ion especially in higher oxidation states it has high affinity to O donor binding sites. Organic and inorganic phosphates are essential vanadium binders both in extracellular and intracellular fluids. Adenosine-5'-phosphates (AMP, ADP and ATP) as the must widely spread organophosphates in living systems, are suspected to be among the most important vanadium binders. Vanadium – phosphate interactions may help stabilizing the V(IV) oxidation state *via* trapping of oxovanadium by chelating phosphate moieties.

Comparative solution speciation studies have been carried out to clarify the VO(IV) binding ability of adenosine-nucleotides and inorganic diphosphate and triphosphate. pH-Potentiometry has been used to determine the stoichiometries and stabilities of the complexes formed and spectral (electronic absorption and ESR) measurements to establish their binding characteristics.

Inorganic phosphates were found to form various mono and bis chelated (normal, partly protonated or deprotonated/hydroxo) complexes. Besides, a trinuclear diphosphate bridged trimer is formed almost exclusively in the pH-range 3-8 in the VO(IV)-$P_2O_7^{4-}$ system; this species, however, could not be detected in the VO(IV)-$P_3O_{10}^{5-}$ system. The reason probably lies in electrostatic and/or steric effects which hinder the aggregation of the highly charged monomeric VOA^{3-} units. The phosphate moieties are the basic binding sites for VO(IV) in adenosine-nucleotides, too. At pH > 10, however, ribose alcoholates become more effective binding sites and the stepwise deprotonation of the phosphate coordinated species is accompanied by structural rearrangements from phosphate coordination to ribose-like bonding mode.

In AMP, besides monodentate phosphate coordination the additional binding of the nucleobase N7 donor atom is also possible; the formation of a dimeric species $(VO)_2A_2$ is stabilised by the stacking interaction between the aromatic adenine rings.

CONFORMATIONAL ALTERATION OF ALCOHOL DEHYDROGENASE UPON PQQ BINDING

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Alcohol dehydrogenase[1] from C. testeroni is a monomeric protein (67 kD) capable of enantioselective reduction of alcohols. The enzyme can be isolated in either the apo or the holo-form, which means that one PQQ molecule per monomer must be added in order to obtain the active enzyme. The protein contains also one heme c and requires the presence of calcium to induce cofactor association and enzymatic activity.

The chemical shifts of the heme methyl groups observed in the ^{1}H-NMR spectrum were shown to be very sensitive to the binding of the PQQ cofactor. The conversion of the apo to the holo-form induces an increase of +7.55 ppm in the chemical shift of one of the methyl groups, while the others are also affected, resulting in an average shift of +2.55 ppm per methyl group. The influence of several metal ions was tested as calcium substitutes, but only minor chemical shifts and linewitdth variations were observed.

EPR spectra of the two forms show only a small g-value change, but a large decrease of the linewidth in the holo-form.

Electronic spectral bands change to higher wavelengths upon PQQ binding.

The redox titrations of the two forms indicate that the holo-form is +60 mV more positive than the apo-form.

Reconstitution of the apo-protein upon titration with PQQ revealed that the two forms are in slow chemical equilibrium ($<3 \times 10^2$ s^{-1}) and that the apo-form has a very high affinity to the cofactor ($K_a > 10^6$).

The absolute necessity of the PQQ for activity, the heme redox potential changes detected and the spectroscopic observations suggest that an important protein conformational alteration takes place upon PQQ binding.

1 Groen, B. W., van Kleef, M. A. G. & Duine, J. A. (1986) Biochem J. 234, 611-615

OXIDO-REDUCTASE ACTIVITY OF A BACTERIAL REDOX CHAIN AND OF ENGINEERED CU SITES

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The present contribution consists of two parts. The first part deals with the genetics, spectroscopy and kinetics of a bacterial redox chain, the second part deals with the engineering of Cu sites in oxido-reductases.

The flow of electrons along the methylamine dehydrogenase redox chain of *Thiobacillus versutus* starts with the enzyme methylamine dehydrogenase (MADH), continues along the blue copper protein amicyanin and the heme containing cytochrome-c550, and ends with a cytochrome aa_3 oxidase for which oxygen functions as the terminal electron acceptor. The MADH and amicyanin genes appear to be located on a cluster containing about 10-12 open reading frames. The organisation of the cluster and the significance of some of the ORF's for the processing of the MADH co-factor (di-tryptoquinone) will be discussed. The 3D-structure of the amicyanin has been determined independently by X-ray diffraction and multi-dimensional homo- and hetero-nuclear NMR methods. The solution structure of the active site appears to be pH dependent and this has important consequences for the redox activity and midpoint potential of the amicyanin. The cyt-c550 has been brought to heterologous expression in *E. coli* and subjected to site-directed mutagenesis of surface residues and of the axial ligands of the heme. Charged surface residues appear not to be critical for the cyt-c to function as the redox partner of the amicyanin. The axial ligand mutations affect the redox properties of the protein as well as the peculiarities of the 'alkaline transition'. A detailed study of the kinetics of the electron exchange between MADH and cyt-c550 in the presence and absence of Zn^{2+} and Ag^{1+} substituted amicyanin has revealed that the strength of the association between MADH and amicyanin is probably dependent on the redox state of the amicyanin. The implications for the redox mechanism will be discussed.

As regards the natural variety of metal sites in Cu containing oxido-reductases, it has been a challenge to understand how the characteristic features of the various types of site are connected with differences in mechanistic properties. Much progress in this field is due to the implementation of site-directed mutagenesis techniques to modify metal sites. The possibilities to engineer the metal sites of the small blue copper proteins will be illustrated by discussing the properties of azurin mutants in which His46, His117 and Met121 have been replaced by other residues. The possibility to convert a type 1 into a type 2 site and vice versa will be demonstrated. The construction of a Cu site in which the metal is coordinated by (among others) 3 histidines, or a site in which various externally added ligands enter the coordination sphere of the Cu, like Cl⁻, may have relevance for the discussion on the structure of the Cu_B site in the cytochrome oxidases and the proton pumping mechanism employed by the latter enzyme.

MECHANISM OF NADPH OXIDATION CATALYSED BY HUMAN MYELOPEROXIDASE: REQUIREMENT OF A MEDIATING MOLECULE.

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Reactions catalysed by myeloperoxidase appear to make major contributions to the microbicidal action of human polymorphonuclear leucocytes. Their activation is associated with a substantial increase in oxygen uptake, an increase in glucose oxidation and a decrease in the NADPH/NADP$^+$ ratio. In the present work we have studied the oxidation of NADPH by the human myeloperoxidase (MPO) / H_2O_2 system and tried to characterize its mechanism. Using steady-state kinetics data show that the reactions of NADPH oxidation by MPO/H_2O_2 require the presence of a mediating molecule (phenolic compounds, ABTS, NaCl) which is directly oxidized by the MPO/H_2O_2 system. We have used spectral studies to identify the roles of the different H_2O_2/myeloperoxidase compounds in NADPH oxidation. Data indicate that the mediating molecule performs one electron reduction of compound I and compound II at a rate faster than NADPH alone. In the overall reaction the limiting step is identified at the level of the reduction of compound II back to native enzyme. Efects of Super Oxide Dismutase on NADPH oxidation reveal differences in the participation of superoxide to the reaction. In conclusion the reaction mechanism and the resulting reaction product from NADPH oxidation (defined with or without coenzyme activity in the glucose 6 phosphate dehydrogenase system) depend on the intermediary oxidation state of the mediating molecule.

Mechanistic Studies of Platinum(II) Complexes Containing Iminoethers: a *trans* Platinum Antitumor Agent.

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For many years, cis-$[PtCl_2(NH_3)_2]$ has been successfully used as a chemotherapeutic agent against testicular, ovarian, cervical, bladder, head and neck tumors. Unfortunately, cis-DDP has only minor activity against common malignancies, such as colon and breast cancers, and its efficacy is limited by a variety of adverse effects.

The search for new clinically more efficacious platinum compounds, on the basis of the structure-activity relationships valid for cis-DDP, has generally been unsuccessful. Recently, new active platinum complexes with structural features that violate the "classical" structure-activity relationships have been described. Non-neutral compounds of the form $[PtCl(NH_3)_2(Am)]^+$ and $[\{trans\text{-}PtCl(NH_3)_2\}_2\{H_2N(CH_2)_nNH_2\}]^{2+}$ and *trans* complexes of formula $trans$-$[PtCl_2(L)(L')]$ have shown considerable cytotoxic effects in murine and human tumor cell lines.

We have investigated the effect of substitution of iminoethers for ammines in *cis*- and *trans*-DDP. Iminoethers, like amines, are potential N-donor ligands and the nitrogen atom carries a hydrogen atom suitable for hydrogen-bond formation. In addition to the *cis* and *trans* geometry, platinum-iminoether complexes can have either E or Z configuration about the C=N double bond of the iminoether ligands, and this brings in another feature to be considered for structure-activity relationships.

A preliminary investigation[1] of the biological activity of the platinum-iminoether complexes had shown that: a) The *in vitro* inhibition of cell proliferation (P388 leukemia) was smaller for *cis* than for *trans* isomers in the case of iminoether complexes while it was much greater for *cis* than for *trans* isomers in the case of ammine complexes. b) The *in vivo* antileukemic activity was significantly greater for *trans-EE* than for *cis-EE* and, unlike *cis-EE*, *trans-EE* was effective also on a cisplatin-resistant subline of P388 leukemia.

In this study we have extended the antitumoral investigation of *trans-EE* to the Lewis lung carcinoma system, and undertaken mechanistic studies in order to address the chemical and biological properties of the new compounds. In particular, some biological effects (inhibition of DNA synthesis and mutagenicity) and interaction with DNA (binding affinity, secondary structure modification, interstrand cross-link formation) have been studied in comparison with those of *cis*-and *trans*-DDP.

All the results point to a mechanism of action of the *trans* iminoether complex different from that of cisplatin.

[1]M. Coluccia, A. Nassi, F. Loseto, A. Boccarelli, M.A. Mariggiò, D. Giordano, F.P. Intini, P. Caputo and G. Natile, *J. Med. Chem.*, **1993**,*36*, 510.

EPR STUDIES ON THE NITRATE REDUCTASE
FROM *Pseudomonas (Ps.) nautica.*

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Ps. nautica 617 is a Gram - negative bacterium isolated from a marine sediment and considered a true denitrifier able to carry out the complete reduction of nitrate to dinitrogen. Nitrate reductase, a membrane bound enzyme that reduces nitrate to nitrite, has been solubilized by heat and purified to homogeneity. The enzyme is composed by two subunits, (120 and 42 kDa) and contains iron-sulfur centers and molybdenum (the nitrate redution site) and is associated with a cytochrome *b* (electron carrier) [2].

In the native state, at 40 K, a mixture of Mo(V) EPR signals was observed (designated as high and low pH forms in other nitrate reductases [3]). At low temperature (below 10 K), it was possible to observe an isotropic signal centered at g=2.02, assigned to a [3Fe-4S] center. Upon reduction, complex iron-sulfur center EPR signals were detected with g-values at 2.051, 1.948, 1.900 and 1.874.

1 - G. Fauque, J. J. G. Moura, S. Besson, L. Saraiva and I. Moura, (1992), *Océanis* **18**, 211-216.
2 - T. Brittain, R. Blackmore, C. Greenwood and A. J. Thomson, (1992), *Eur. J. Biochem.*, **209**, 793-802.
3 - C. Godfrey, C. Greenwood, A. J. Thomson, R. C. Bray and G. N. George, (1984), *Biochem. J.*, **224**, 601-608.

MODELING THE DIOXYGEN BINDING SITE OF HEMOCYANIN WITH THE APPROXIMATE DENSITY FUNCTIONAL THEORY

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Hemocyanins are large multisubunit proteins that transport molecular oxygen in a variety of invertebrates with molecular weights up to 3,500,000. Recently the three-dimensional structures of deoxygenated hexameric hemocyanins from *Panulirus interruptus* and of the oxygenated form of the hemocyanin subunit II of *Limulus poliphemus* were refined at atomic resolution (3.2 Å and 2.4 Å, respectively).

So far two different computational methods have been used for modeling the copper-peroxide complex: semiempirical SCF-$X\alpha$-SW calculations (broken-symmetry self-consistent field-$X\alpha$-scattered wave) calibrated through comparison with spectral data (Ref.5), and *ab initio* computations (at different levels including restricted closed shell (RHF), high spin restricted open shell (RHOF), generalized valence bond wavefunctions (GVB) and configuration interaction (CI)).

In the present work, the relevance of the metal ligands with respect to the electronic structure of the active site is investigated. We use a functional density approach, because of its computational expedience in treating large size molecules as compared to classical *ab initio* calculations. We focus on four different models of the oxygen binding site, characterized by the μ-η^2:η^2 geometry of the peroxo-copper complex and different with respect to the number and complexity of metal ligands. Our results indicate that the approximate density functional theory is sufficient to describe the main physical features of the oxyhemocyanin binding site in the ground state when at least four metal ligands are included in the model.

Metal Complexes of Sulfanilamide Derivatives. Synthesis, crystal structure of $CuCl_2[Cu_2(stz)_4]H_2O$, a complex with copper(II) dimer and copper(II) monomer. Superoxide Dismutase mimetic activity.

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Sulfanilamide and its N-substituted derivatives are well-known antibacterial drugs. The metal complexes of these substances are extensively studied, Bult[1] has classified the metal complexes of sulfanilamide and its N-substituted derivatives in four classes: a) $M(HL)_mX_{n'}$, where the sulfanilamide HL is present in its neutral form. b) ML_m, where the sulfanilamide HL is deprotonated. c) $(H_2L)_2[MCl_4]$, where the sulfanilamide is protonated and present as counterion. d) ML_mY_n where Y is a suitable solvent or a base such ammonia, morpholine, imidazole, pyridine etc.

We have undertaken several studies on the characterization of the metal complexes of sulfanilamides N-substituted, mainly the sulfatiazole (Hstz):

$$H_2N-\!\!\!\bigcirc\!\!\!-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-\underset{\underset{H}{|}}{N}-\!\!\bigcirc\!\!$$
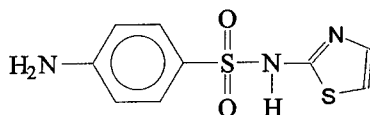
In early studies[2,3] we have reported the synthesis and structural characterization of $Zn(stz)_2.H_2O$ and $[Cu(Hstz)_2(MeOH)Cl_2]$ complexes. The present paper describes the crystal structure of an copper(II)-sulfathiazole complex including in the class b) of the classification of Bult, but with a singular formula: $CuCl_2[Cu_2(stz)_4]H_2O$. The dimer units $Cu_2(stz)_4$ are linked by means monomeric $CuCl_2$ units. The superoxide-dismutase mimetic activity of the complex is compared with that of other related copper complexes and with that of the enzyme.

1.- Bult A., *Metal Complexes of Sulfanilamides*, Vol. 16 in *Metal Ions in Biological Systems*, Sigel Ed., New York, 1983.
2.- Casanova J, Alzuet G., Ferrer S., Borrás J., García-Granda S. and Perez-Carreño E., *J. Inorg. Biochem.*, **51**, 689 (1993).
3.- Casanova J., Alzuet G., Borrás J., David L. and Gatteschi D., *Inorg. Chim. Acta*, **211**, 183 (1993).

BINDING OF SMALL MOLECULES TO TRINUCLEAR AND DINUCLEAR SITES OF COPPER PROTEINS

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The investigation of the binding of small exogenous molecules to the active site of multicentre proteins can give important information on the geometric and electronic structures of the metal clusters, particularly when the ligands act as inhibitors of the protein activity. A number of catalytic and spectroscopic studies on the effect of anions like azide and fluoride have been performed, in particular, in the case of copper proteins.[1] These two ions inhibit the activity of ascorbate oxidase competitively with respect to ascorbate and noncompetitively with respect to dioxygen,[2] and greatly accelerate the formation of the met derivative of hemocyanin, which no longer binds dioxygen.[3] It has been proposed that azide[4] and fluoride[4a,5] bind as bridging ligands between the Type 2 and Type 3 centres of ascorbate oxidase, and that azide is also bridging to the dinuclear site of met-hemocyanin.[6]

In the present investigation NMR relaxation measurements have been used, together with other spectroscopic techniques, to study the complexes between azide or fluoride and the proteins. The titration of $^{15}N_3^-$ or $^{19}F^-$ with ascorbate oxidase causes an enhancement in the longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates, indicating a strong interaction between the paramagnetic centres and the inhibitors. The paramagnetic contributions to the relaxation (T_{1M}, T_{2M}) are different when the enzyme is in the native form or in the Type 2 copper-depleted form . The values obtained in the latter case are very similar with those obtained employing hemocyanin. The presence of ionizable groups which are important for the binding of the exogenous anions has also been established by investigating the effect of pH.

1. *Bioinorganic Chemistry of Copper*,K.D. Karlin,Z. Tyeklàr eds.,Chapman & Hall, N.Y., 1993.
2. R.E. Strothkamp, C.R. Dawson, *Biochemistry*, **16**, 1926, 1993.
3. R. Witters, R. Lontie, *FEBS Letters*, **60**, 400, 1975.
4. (a) J.L. Cole, L. Avigliano, L. Morpurgo, E.I. Solomon, *J. Am. Chem. Soc.*, **113**, 9080, 1991. (b) L. Casella, M. Gullotti, G. Pallanza, A. Pintar, A. Marchesini, *Biol. Metals*, **4**, 81, 1991.
5. J.H. Dawson, D.H. Dooley, H.B. Gray, *Proc. Natl. Acad. Sci. USA*, **77**, 5028, 1980.
6. F. Tuczek, E.I. Solomon, *Inorg. Chem.*, **32**, 2850, 1993.

STEREOCHEMICAL CONTROL IN CHLOROPEROXIDASE CATALYZED REACTIONS

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Chloroperoxidase is a rather versatile enzyme since it can catalyze classical reactions of peroxidases, catalatic type reactions, oxidative halogenations and oxygen-transfer reactions which are more typical of cytochrome P-450. This versatility depends on the particular structure of the active site of the enzyme, with a cysteine thiolate as the heme axial ligand and a polar heme environment, which appears to be similar to that of common peroxidases but, unlike these enzymes, allows the access of substrates to the distal pocket. This accounts for the marked stereoselectivity effects observed in the catalytic oxidation of organic sulfides and the epoxidation of olefins. The reactivity of chloroperoxidase toward a series of phenolic compounds also exhibits marked selectivity effects. These operate through charge, preventing phenolic compounds carrying amino groups on the substituent chain to act as substrates for the enzyme, and through size, excluding potential substrates containing bulky substituents to the phenol nucleus. The characterization of chloroperoxidase-substrate complexes with a variety of spectroscopic techniques, including paramagnetic NMR and relaxation rate measurements, shows that binding of substrates occurs close to the heme in the distal pocket. Phenolic substrates bind with the phenol group pointing toward the iron atom, whereas phenolic compounds which are not substrates bind to the enzyme with a much different disposition, with the phenol group very distant from the iron and probably actually outside the active-site cavity.

KINETIC STUDIES ON THE INTERACTION BETWEEN CYTOCHROME C_3 AND FLAVODOXIN FROM *Desulfovibrio gigas*

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Cytochrome c_3 from *Desulfovibrio gigas* is a small (MW=13kDa) tetraheme protein which exhibits a cooperative behaviour. NMR studies on this protein have shown that the four hemes and an acid-base group constitute an interdependent consortium: the heme redox potentials depend on the pH (redox-Bohr effect) and during a redox titration process interactions among different hemes occur, thus changing the values of redox potentials according to which of the hemes are oxidised (redox interacting potentials) [1, 2]. The kinetics of reduction of this cytochrome by sodium dithionite were studied using stopped-flow techniques and showed a biphasic pattern with a fast phase accounting for ca. 25% of the total absorbance change, indicating that heme 4 in the sequence (the heme with the least negative redox potential) reacts faster than the other three hemes [3]. Although this heme is the less exposed to the solvent, it is the nearest one to the lysine patch possibly used for the docking of the physiological partners [4]. Kinetic studies on the interaction between cytochrome c_3 and flavodoxin from *D. gigas* are presented and compared to those of the reduction of flavodoxin and FMN by sodium dithionite.

[1] H. Santos, J.J.G. Moura, I. Moura, J. LeGall and A.V. Xavier, *Eur.J.Biochem.* **141**, 283 (1984)

[2] M. Coletta, T. Catarino, J. LeGall and A.V. Xavier, *Eur.J.Biochem.* **202**, 1101 (1991)

[3] T. Catarino, M. Coletta, J. LeGall and A.V. Xavier, *Eur.J.Biochem.* **202**, 1107 (1991)

[4] D.E. Stewart, J. LeGall, I. Moura, J.J.G. Moura H.D.Jr. Peck A.V. Xavier, P.K. Weiner and J.E Wampler, *Biochemistry* **27**, 2444 (1988)

SYNTHESIS, CHARACTERIZATION AND DEAMINATION OF AN N-SALICYLIDENEGLYCYLGLYCINATO OXOVANADIUM(IV) COMPLEX

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Several published studies concern the preparation and reactivity of vanadium complexes of N-salicylideneamino acids[1]: these may be considered as model systems for some reactions of pyridoxal-potentiated enzymes. However to our knowledge, no such studies with simple peptides have been reported.

The present communication deals with the preparation and characterization by elemental analysis, thermal (TG and DSC), magnetic and spectroscopic methods, of a solid formulated as $VO(sal-glygly)(H_2O)_n$ (sal-glygly = N-salicylideneglycyl-glycinate; n=1.5-3.0). From similar but dilute solutions the decavanadate, $(NH_4)_4(Na)_2[V_{10}O_{28}].10\ H_2O$, was isolated after ageing: its structure has been determined by X-ray diffraction analysis. Apparently the same decavanadate was obtained from similar dilute solutions which were designed to yield $[VO(sal-thr)(H_2O)]$ but a different polyvanadate from solutions containing $[VO(sal-ala)(H_2O)]$ (sal-thr = N-salicylidene-S-threoninate and sal-ala = N-salicylidene -S-alaninate).

The formation of a compound of vanadium(V) may be explained by the oxidation of oxovanadium(IV) by atmospheric oxygen. The NH_4^+ cations formed by deamination of the glygly present in solution. This process may have involved an oxidative deamination catalysed by salicylaldehyde and the metal ion.

References

1. I. Cavaco, J. Costa Pessoa, D. Costa, M.T. Duarte, R.D. Gillard and P.M. Matias, *J. Chem. Soc. Dalton Trans.* 1994, 149 and references therein.

TIN(IV) COMPLEXES WITH THIONICOTINIC ACID DERIVATIVES AS POSSIBLE ANTITUMOR AGENTS

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Following our previous researches on inorganic and organo-Tin complexes as potential antitumor agents, we have prepared a new series of complexes with mercaptopyridines. The main aim of this work is to compare the chemical and biological properties of inorganic Tin complexes with those of the organometallic analogues in the mainframe of their structure-activity relationship.

The present note reports complexes with N-alkyl esters of thionicotinic acid. Compounds with different stoichiometries have been obtained as a consequence of the ability of the ligand to act as a neutral or as a monoanion agent. All the prepared compounds have been characterized by means of infrared and Mössbauer spectroscopies. On the basis of the information so obtained, an octahedral structure may be proposed for all the complexes with the ligands behaving in a mono- or bi-dentate way.

"In vitro" experiments to investigate their cytotoxicity on tumor cell lines are in progress.

Ceruloplasmin-assisted incorporation of Fe(III) into apoferritin.

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Ferritin stores iron in a soluble form by accomodating it as a ferrihydrite micelle in the cavity of the apoferritin shell. Natural apoferritins are heteropolymers composed of H and L chains in various proportions; the H chains contain a ferroxidase center and promote iron oxidation, the L chains iron accumulation. "In vitro" iron uptake takes place when Fe(II) is furnished to apoferritin in the presence of oxygen, whereas little iron, if any, is incorporated when it is furnished as Fe(III).

Ceruloplasmin (CP), a blue-copper oxidase, has been reported to increase the rate of iron uptake in horse spleen apoferritin (90% L); however, it was not established whether its action is due to direct transfer of Fe(III) to the apoferritin molecule. We measured the kinetics of iron incorporation into horse spleen apoferritin, H- and L-homopolymers and observed that the effect of CP on the rate of iron uptake parallels the pH and NaCl concentration dependence of the enzyme activity. Moreover, CP permits complete uptake of iron into the L-homopolymer under conditions in which no iron is taken up in the absence of the enzyme. This finding provides unequivocal evidence that CP-assisted iron incorporation entails direct transfer of Fe(III) to apoferritin.

The use of EPR spectroscopy to follow incorporation of iron into apoferritin in the presence of ceruloplasmin reveals the formation of mononuclear Fe(III) concomitant with the quenching of type 1 copper resonances. In horse spleen apoferritin and in the H-homopolymer, but not in the L-homopolymer, a radical signal at g=2 also appears albeit with a slower kinetics. The rate of appearance of the g=2 signal is not consistent with the formation of the Fe(III)-tyrosinate adduct described by Waldo et al. (1993), indicating that the radical is likely to be formed upon rearrangement of the Fe(III)-apoferritin complex.

NEW ISOMERS OF PORPHYRINS AND METALLOPORPHYRINS: METHYLATION OF TETRAPHENYLPORPHYRIN WITH INVERTED PYRROLE RING

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2-aza-21-carba-5,10,15,20-tetraphenylporphyrin (CTPP), recently discovered[1] isomer of TPP, having one of the pyrrole rings inverted preserves the general pattern of macrocyclic structure and aromatic character. As the consequence of the pyrrole inversion, the coordination core of CTPP consists of three nitrogen donors and one carbon atom which coordinate readily to nickel(II) ion giving diamagnetic, strictly planar NiCTPP [1]. Strongly nucleophilic character and spatial disposition of the outer nitrogen of CTPP promote its methylation which undergoes highly selectively giving 2-methyl-2-aza-21-carba-TPP (MeCTPP). Ni(II)MeCTPP, a green diamagnetic complex is formed in mild conditions, which implies labilization of the inner C-H bond.
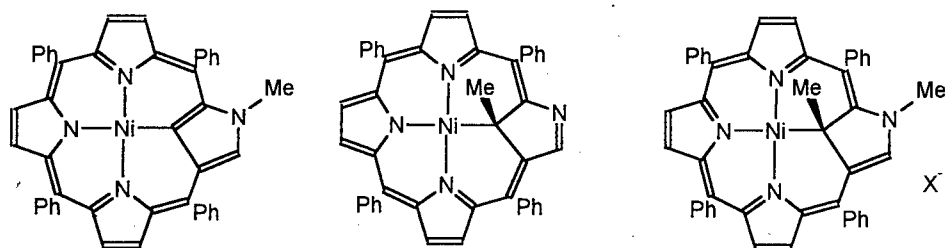
Methylation carried out for NiCTPP leads in the first step to the formation of a complex having a CH_3 group placed inside the macrocyclic ring bound to the coordinated carbon atom. This paramagnetic complex can be converted reversibly to the diamagnetic species by simultaneous abstracting of proton from the outer nitrogen and an axial ligand coordinated to the metal center. In the second step of methylation the methyl group is placed on the outer nitrogen. All but one ^{1}H NMR pyrrole proton signals of this paramagnetic, double methylated species are situated downfield and can be connected in pairs by COSY experiment.

1. P.J.Chmielewski, L.Latos-Grażyński, K.Rachlewicz, and T.Głowiak, *Angew.Chem.* 1994, *106*, 805.

Oligonucleotide studies of the kinetics and selectivity of DNA platination.

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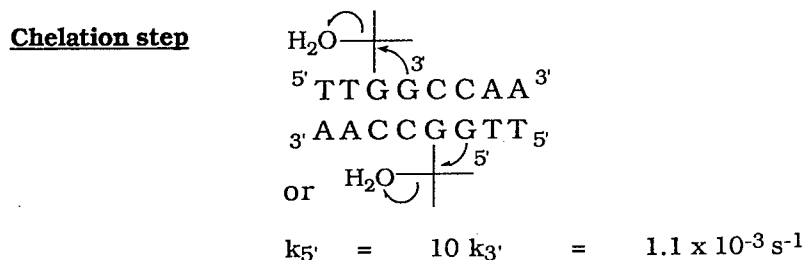
With the aim to design sequence and nucleotide specific drugs, we wish to determine which factors control the kinetics and selectivity of DNA platination.

The kinetic work was carried out for the platination of d(TGG), d(TTGG), d(CTGGCTCA), d(TTGGCCAA)$_2$ by : $[Pt(NH_3)_3(H_2O)]^{2+}$, $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[PtCl(NH_3)_2(H_2O)]^+$, based on an HPLC analysis of the reaction mixture, including the 5' and 3' intermediate monoadducts of the GG sequence.

The results for the duplex structure are the following :

$[Pt(NH_3)_3(H_2O)]^{2+}$	$k_{5'}$ =	5 $k_{3'}$	=	4.5 $M^{-1}s^{-1}$
$[Pt(NH_3)_2(H_2O)_2]^{2+}$	$k_{5'}$ =	15 $k_{3'}$	=	32 $M^{-1}s^{-1}$
$[PtCl(NH_3)(H_2O)]^+$	$k_{5'}$ =	$k_{3'}$	=	0.2 $M^{-1}s^{-1}$

Chelation step

$$k_{5'} = 10\, k_{3'} = 1.1 \times 10^{-3}\, s^{-1}$$

The selectivities observed for the duplex structure will be compared to those found on the single strands. They will be discussed on the basis of a molecular modeling analysis of the pentacoordinated intermediates of the first platination step.

TOTAL SYNTHESIS AND SPECTRAL CHARACTERIZATION OF
D. GIGAS RUBREDOXIN AND RUBREDOXIN FRAGMENTS

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Total chemical synthesis of small, natural metalloproteins is becoming feasible[1,2]. This holds interesting perspectives for mapping reactivity and electronic structure of the metal and factors important in the three-dimensional protein structure. As a group of small (50-55 residues), well-characterized iron/sulphur proteins the Rubredoxins (Rd) are good targets for chemical metalloprotein synthesis. The 5-12 and 35-50 segments are, moreover highly conserved while the intermediate (13-34) segments constitute a loop remote from iron and are much less homologous (fig.1). This holds particular promises for identification of the molecular folding stablity factors by chemical synthesis.

We have accomplished total synthesis of *D. gigas* Rd (52 residues) by solid-phase peptide synthesis and peptide folding around Fe(II). The synthetic Rd was purified by HPLC and ultrafiltration. The protein is stable, oxidizes and reduces reversibly, and UV/VIS, CD, resonance Raman and MALDI-MS mass spectrometry show *in detail* all the expected features (fig.2). In comparison, a 25-residue fragment and its Val41 → Leu variant in which the 13-34 loop and 1-4 terminal are cut and Val5-Glu50 tied by Gly folds to correct conformation as revealed by UV/VIS and MALDI-MS spectrometry, but is susceptible to both loss of metal and irreversible oxidation[3].

The results show that total synthesis of long peptides, correct peptide folding, and an electronically functional totally synthetic metalloprotein have been achieved. They also show that residues remote from the metal are important for a stable three-dimensional protein structure.
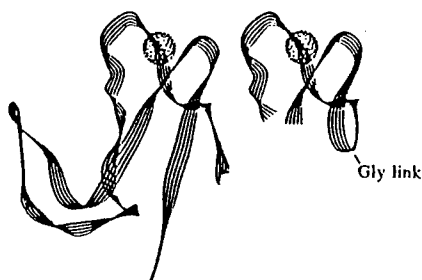

Fig.1. Three-dimensional structure of *D.gigas* Rd (left) and calculated structure of 25-residue Rd analogue (right).
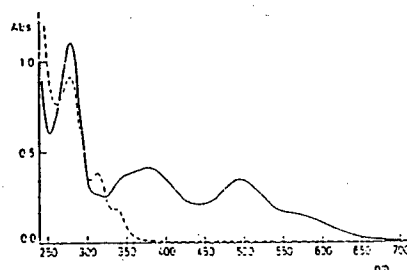
Fig.2. UV/VIS spectra of oxidized (fully drawn) and reduced (dashed) totally synthetic Rd ($\approx$ 48 μM).

1. E.T. Smith and B.A. Feinberg, *J. Am. Chem. Soc. 113* (1991) 688.
2. L.E. Zawadzke and J.M. Berg, *J. Am. Chem. Soc. 114* (1992) 4002.
3. H.E.M. Christensen, J.M. Hammerstad-Pedersen, A. Holm, P. Roepstorff, J. Ulstrup, O. Vorm and S. Østergaard, *FEBS Lett. 312* (1992) 219.

^{1}H NMR studies of the newly discovered HiPIP from *Rhodoferax fermentans*

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The ^{1}H NMR spectra of the newly discovered high potential iron-sulfur protein (HiPIP) from *Rhodoferax fermentans* [1] have been recorded for the oxidized and reduced forms of the protein. The spectra are very similar to those observed for *Rhodocyclus gelatinosus* HiPIP. The temperature dependence of the hyperfine shifted signals in the reduced form of the HiPIP has been simulated using a simple Heisenberg spin Hamiltonian and considering a completely delocalized electronic structure. From this calculation we obtain a value of $J = 300$ cm^{-1} as a reasonable estimation of the average magnetic coupling constant, as well as values for the hyperfine coupling constants for the individual protons. From the latter values a simulation of the temperature dependence of the oxidized protein has been performed. We find that the ratios of the hyperfine constants values within each β-CH$_2$ pair is maintained, within experimental uncertainties, for all pairs except for the β-CH$_2$ pair of Cys 43 (*C. vinosum* numbering). This confirms earlier observations [2] that Cys 43 undergoes a change in orientation upon oxidation, and that the difference, sizable at room temperature, further increases with increasing temperature.

[1] Ciurli, S.; Hochkeoppler, A.; Ferro, P.; Zannoni, D. submitted
[2] Bertini, I.; Capozzi, F.; Ciurli, S.; Luchinat, C.; Messori, L.; Piccioli, M. *J. Am. Chem. Soc.* **1992**, *114*, 3332-3340.

Superparamagnetic Iron Oxides Nanoparticles as Contrast Agents for Magnetic Resonance Imaging

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Superparamagnetic iron oxides nanoparticles (SPIO), have been extensively studied over the past few years, from their relaxation properties to their clinical applications for Magnetic Resonance Imaging of the liver, spleen and lymph nodes.

The aim of this talk is to explain how the high r_1 and r_2 relaxivities of the particles are responsible for potential positive and negative signal enhancement, depending on the dose injected, the sequence used and their biological status (free or clustered).

Since they were first described in the literature, these particles have been mainly used in clinical trials or experimental studies for their powerful negative enhancement properties induced by their high r_2 relaxivity and susceptibility effects. However, there are now many experimental and clinical studies showing that iron oxides particles can induce positive enhancement (brightening). Thus, the dual enhancement properties of these particles have to be considered in order to avoid image interpretation pitfalls, as well as new potential applications.

The influence of the particles biodistribution on signal enhancement will be discussed with a special focus on three parameters: vascular space distribution, tissue uptake capabilities, and capillary permeability.The important parameters governing contrast can be summarized that way:

- A positive enhancement can be observed either in vitro or in vivo only when using T1-weighted sequences and with a low dose of iron oxides.

- In vivo, the contrast pattern is mainly dose-dependent when there is non active uptake of the particles as in the vascular space or in tissues such as the myocardium.

- In the presence of an active particle uptake, a positive enhancement can be observed in the liver within a short delay after injection of a low dose. After that, the uptaken particles induce a strong negative enhancement through a magnetic susceptibility mechanism.

The basis of uptake, perfusion and capillary leak imaging using SPIO are then drawn. Based on the results of recent clinical trials in liver and lymph node imaging, the implications of these physico-biological properties on image analysis are explained and illustrated.

Oxidation Level Studies of Metal Ene-1,2-dithiolate Centres

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The ene-1,2-dithiolate chelating ligand is postulated to be present in the molybdopterin cofactor of the oxo-transfer molybdoenzymes. Also, we have recently shown that a nickel complex of the prototypical quinoxaline-2,3-dithiolato (qdt) ligand can catalyse the production of hydrogen from protons under reducing conditions, therefore forming a model for the nickel site in the hydrogenase enzymes. The description of the oxidation levels of complexes containing 'non-innocent' ligands has continued to be the subject of much debate since their discovery in the 1960's and we are applying a range of spectroscopic techniques to probe the electronic structure of these biologically important model systems.

L- and K-edge X-ray absorption spectroscopy (XAS) have been used as complementary probes of the bound state transitions in a range of nickel complexes of the general formulae: $[Ni(qdt^*)_2]^{n-}$, where n = 1 or 2 and qdt* is variously substituted at the 6 and/or 7 position; $[Ni(qdt^*)(P-P)]$, where P-P = chelating phosphine. EPR and ESEEM spectroscopy have been used to determine the extent of the unpaired electron density for the complexes $[M(qdt^*)_2]^{n-}$, where n=1, M = Ni and n = 2, M = Cu. The complexes have contained the atoms in natural abundance as well as enriched by ^{61}Ni, ^{13}C and for the substitution of S by Se. Reactive intermediates have been probed by the use of *in situ* spectroelectrochemistry.

Our results show that (i) redox processes and catalytic activity are particularly sensitive to the substitution on the benzene ring of qdt*; (ii) bound state transitions at sulphur in XAS are affected by the formal oxidation state of the metal; (iii) the unpaired electron density of the pyrazine ring of qdt* is dependent on the orbital population at the metal; (iv) electronic transitions at the metal L-edge are determined by the coordination environment at the metal.

Mechanistic studies on platinum(II) complexes with imino ethers: a *trans*-platinum antitumor agent.

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Cis and *trans* complexes of formula $[PtCl_2\{HN=C(OMe)Me\}_2]$ have been prepared by hydrolysis of the corresponding nitriles and their biological activity has been examined and compared with their NH_3 analogues, *cis*- and *trans*-DDP. The imino ether ligands can have E or Z configuration about the CN double bond, therefore EE, EZ and ZZ isomers are obtainable. Substitution of imino ether for ammine determines a dramatic change of the biological activity with respect to the *cis* and *trans* geometry. *Trans-EE* shows an antileukemic activity (murine P388 leukemia) higher than *cis-EE* either *in vitro* (ID_{90} = 7.4 and 50 μM, respectively) or *in vivo* (%T/C = 170 and 144, respectively). *Trans-EE* is also active against a P388-subline resistant to *cis*-DDP (%T/C = 133) and shows an efficacy comparable to that of *cis*-DDP in reducing the primary tumor mass and spontaneous metastases in mice bearing Lewis lung carcinoma. The solvolytic behavior, the DNA interaction properties and cellular effects of platinum imino ether complexes have been investigated. In the solvolysis reaction, both the geometry of the complex and the configuration of the imino ether ligands are preserved; moreover, similarly to ammines, the imino ether ligands are non-leaving groups. Binding to calf thymus DNA is slower for imino ether complexes than it is for ammine complexes. Unlike *cis-EE*, *trans-EE* does not give the DNA local conformational alterations typical of antitumor-active platinum compounds. Moreover, the cytotoxic activity of *trans-EE* does not appear to depend on DNA interstrand cross-link formation.The inhibition of DNA synthesis and the mutagenic activity also indicate that the behavior of *cis*- and *trans-EE* is reversed with respect to *cis*- and *trans*-DDP, *trans-EE* being clearly more active than *cis-EE*. The results point to a new *trans* platinum antitumor complex with a mechanism of action different from that of *cis*-DDP and classical analogues.

Molecular Modelling of Coordination Compounds: Electronic Effects and Spectroscopy.

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Molecular mechanics is a well established method for a rather accurate and efficient computation of structures of organic compounds. The open-shell electronic structure of transition metal compounds leads to stereoelectronic effects (e.g. Jahn-Teller distortions and trans-influences) which are difficult to predict based on the general molecular mechanics parameterization. These are serious problems if structures and spectroscopic properties of metalloenzymes and low molecular weight model compounds are to be calculated. We recently have developed methods and implemented them into a general molecular mechanics program which allow us to predict the d-orbital directionality and Jahn-Teller distortions. The examples presented include an accurate prediction of trigonal twists of octahedral and tetrahedral twists of square planar geometries, and the prediction of the direction and extent of Jahn-Teller distortions of pseudo-octahedral structures. Applications of these methods are discussed in detail.

A new class of model systems for blue copper proteins

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A new class of ligands (see figure) that along with some related systems are used to mimick the spectroscopic (EPR, UV-vis) and redox properties of blue copper proteins are presented. The experimentally determined properties of the corresponding transition metal compounds are used to develop the parameters for molecular mechanics and angular overlap model calculations, which then will be used for the design of modified blue copper protein model systems.
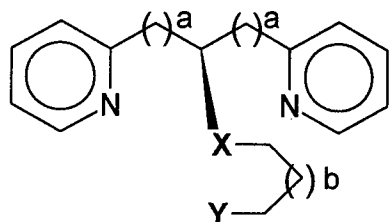
a = 0, 1
b = 0, 1, 2
X, Y = N, S, O

THE CHEMISTRY, STRUCTURE AND SPECTROSCOPIC PROPERTIES OF [N5FeX]+ (X = OCH₂CH₃, OCH₃, Cℓ, SCN) COMPLEXES.

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The iron-oxygen coordination present in non-heme iron proteins, such as hemerythrin, ribonucleotide reductase, purple acid phosphatases, and methane monooxygenase has been of considerable recent interest. The structures of very unsymmetrical compounds [N5FeOFeX₃]Y, where N5 is N-(hydroxyethyl)-N,N',N'-tris(2-benzimidazolylmethyl)-1,2-diaminoethane, where X = Cℓ, Br and Y = Cℓ, Br, NO₃ have been solved by X-ray crystallography. These compounds have strongly enhanced asymmmetric stretch modes v_{as}(Fe-O-Fe) in the infrared compared to Raman and have antiferromagnetic coupling of a magnitude usually only observed in triply bridged diiron compounds and proteins with unsymmetrical dinuclear iron active centers. Attempts to expand the series of compounds, usually lead to the crystallization of [N5Fe(III)OR]Y₂ (R = CH₂CH₃, Y = NO₃), [N5Fe(II)OR]Y₁ (R = CH₃, Y = I (1)) or [N5FeX]Y₂ (X = Cℓ, Y = NO₃; X,Y = SCN) compounds. This work presents the crystal structures of these compounds and examines some of their spectroscopic properties. An investigation in to the chemistry and formation of the μ-oxo species is also reported. Finally, some unusual properties of N5Fe(II) species (2) and their reactions with hydrogen peroxide are presented.
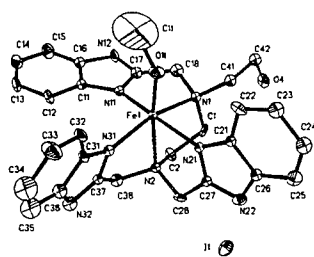
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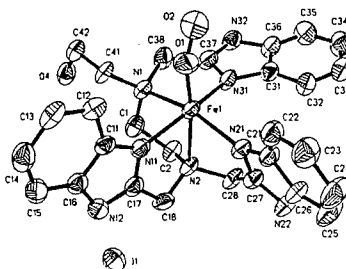
(2)

THE FORMATION OF COMPLEXES IN SOLUTIONS CONTAINING OXOVANADIUM(IV) AND S-HISTIDINE AND RELATED LIGANDS

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Knowledge of the complex equilibria of VO^{2+} in the presence of amino acids is relevant in understanding its possible interaction with likely biological ligands. V.-N(his) bonding has been inferred in some proteins and enzymes. The design of model systems, preferably with S-his as a ligand, and a better knowledge of the coordination modes of this amino acid to VO^{2+} is therefore of interest.

We recently reported[1] studies on the S-histidine + VO^{2+} system in aqueous solution by combining the results of potentiometric and spectroscopic techniques. The objective of the present study is to clarify the structures of species formed in this system. This was done by a comparison of the pH dependence of the visible circular dichroism (CD), visible isotropic absorption (VIS) and electron spin resonance (ESR) spectra obtained for the S-histidine + VO^{2+} system and the corresponding spectra for several S-histidine derivatives and related ligands : 3-methyl-S-histidine, 1-methyl-S-histidine, S-histidine methyl ester, S-histidinol, Nα-acetyl-S-histidine and histamine.

The ESR, CD and VIS spectra of solutions containing 3-methyl-S-histidine and VO^{2+} are similar to those obtained in the S-histidine + VO^{2+} system. Those obtained for solutions containing VO^{2+} and the other histidine derivatives are quite different, notably in the pH range 5-9. This means that in S-histidine complexes in aqueous solution the coordination of the imidazole N, the amino N and the carboxylate O occurs. Comparison of the spectroscopic results for all systems studied indicates plausible coordination geometries.

References

1. J. Costa Pessoa, S.M. Luz, I. Cavaco and R.D. Gillard, 6th International Conference on Bioinorganic Chemistry, San Diego, 1993, U.S.A.

ELECTRON TRANSFER AND STRUCTURAL PROPERTIES
OF CYTOCHROME C_4 AND CYTOCHROME C_3

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Crucial function in photosynthetic and respiratory electron transfer (ET), or in redox metalloenzyme activity, is organized by multicentre proteins. Cooperativity between the centres can emerge from such organization, involving both static electronic or conformational interaction between the centres, and vibrational or conformational coherence between successive ET events.

The ET behaviour of the di-heme cyt c_4 from *P. stutzeri* and the tetra-heme cyt c_3 from *D. gigas* is compared with a view on cooperativity between the hemes. The cyt c_4 crystal structure has been obtained[1] and shows a two-domain dipolar structure. The heme groups are non-coplanar but the propionates within H-bond distance and there is a H-bond network between the domains. The heme potentials are 242 and 328 mV (NHE). The $c^{II}c^{II}$ form is low-spin while low-spin/high-spin equilibrium prevails in $c^{III}c^{III}$. The α-band is split in three components one of which can be assigned to the high-potential heme. The ET kinetics towards $[Co(terpy)_2]^{3+/2+}$ and $[Co(bipy)_3]^{3+/2+}$ is, finally multi-phasic but complete resolution is in principle possible. The ET pattern is indicative of static cooperativity while intermolecular ET is slow.

The four-heme cyt c_3 is structurally well characterized and the cooperative heme redox potentials mapped in detail[3]. The number of microscopic rate constants is prohibitively large for resolution but by the thermodynamic cooperativity mapping and new elements of ET theory the most likely intramolecular ET routes can be identified.

1. A. Kadziola and S. Larsen, Abstract at this conference.
2. J.J. Karlsson, I. Coutinho, A. Kadziola, J. Ulstrup and A.V. Xavier, Abstract at this conference.
2. A.V. Xavier, J. Inorg. Biochem. 28 (1986) 239.

CORRELATION BETWEEN HEME STRUCTURE AND REDOX POTENTIAL IN CYTOCHROME C_3 BY MONITORING THE PSEUDOCONTACT SHIFTS ALONG REDOX TITRATIONS FOLLOWED BY ^{1}H NMR

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The four hemes in cytochrome c_3 from *Dsm baculatum* (MW 13000, His-Fe-His) have midpoint redox potentials, e_i^0, ranging from -400 to -100 mV. The difference between the redox potentials of the two higher-potential hemes is greater than than 100 mV, but between the three lower-potential hemes the e_i^0 differences do not exceed ca. 50 mV. The identification of the higher-potential heme, h4, as HIII in the three-dimensional structure, has been established by following the chemical shift of the heme HIII protons from the fully reduced to the 75% oxidized sample (1). The correlation between the structure and the redox potential of the remaining three hemes is now achieved by comparing the extrinsic pseudocontact shifts undergone by the HIII protons and by the aromatic protons between 0 and 75% sample oxidation with the simulated shift evolutions calculated assuming that: i) the heme core of *Dsm. baculatum* cytochrome c_3 is identical to that of the cytochrome c_3 from *D. vulgaris* (2), ii) the three hemes have identical, axially symetric magnetic susceptibility tensors and iii) $(e_2^0 - e_1^0) = 30$ mV, $(e_3^0 - e_2^0) \in (0, 30)$mV.

(1) I. B. Coutinho, D. L. Turner, J. LeGall and A..V. Xavier
    Biochem. J. (1993) 294, 899-908
(2) I. B. Coutinho, D. L. Turner, J. LeGall and A. V. Xavier
    Eur. J. Biochem. (1992) 209, 329-333

Iron Homeostasis - from in vitro to in vivo

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In order to understand the homeostasis of metal ions within biological systems, we must establish how their uptake, intracellular utilisation, storage and eventually export is regulated. In the case of iron, many in vitro studies have suggested that at least in so far as iron uptake, storage, and in erythroid cells actively involved in haem synthesis, utilisation, the mechanism of homeostasis might involve regulation at the level of translation of messenger RNA's (mRNA's). The mRNA's for the iron storage protein ferritin (Ft), transferrin receptor (TfR), and in erythroid precursors δ-aminolaevulinate synthase (ALA), the first committed enzyme of haem biosynthesis, have conserved stem-loop sequences (IRE's) which can bind a cytoplasmic aconitase (designated IRF) in either a tight or a loose binding mode. When the iron content of cells is low, the IRF binds tightly (with a less than complete 4 Fe/4S cluster) to the IRE's, prevents synthesis of the storaage and utilisation proteins (ferritin and δ-aminolaevulinate synthase) and allows the synthesis of the transferrin receptor. When cells are iron-replete the IRF is in its low affinity form, allows degradation of TfR mRNA by intracellular nucleases, and permits the synthesis of storage and utilisation proteins (Ft and ALA).

We have shown that this hypothesis seems to hold in animals which have been either iron-depleted or iron-loaded (Ward et al., 1994), with the additional caveat that the IRF system seems also to be tuned to the haematological status of the animal. In effect, when iron-loading is induced by a ferrocene derivative, which deposits its iron directly in parenchymal tissues, the IRF response is at best mitigated. In contrast, when iron-loading is induced by iron dextran, which is taken up in the first instance by reticuloendothelial macrophages, and then refluxes its iron to parenchymal cells, accompanied by an increase in transferrin saturation, the IRF response is clear-cut. We will also discuss the way in which reticuloendothelal iron may reflux to the parenchymal cells, explaining perhaps the 'missing link' in cellular iron homeostasis, namely export.

References:

Ward, R.J., Kühn, L.C., Kaldy, P.,Florence, A., Peters, T.J. and Crichton, R.R. Eur. J. Biochem. 220, 927-931 (1994).

^{13}C NMR MAGIC ANGLE SPINNING EXPERIMENTS in IRON - SULFUR CLUSTERS : CASE of the $[Fe_4S_4]^{2+}$ STATE.

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Iron-Sulfur proteins exhibit shifts of the proton and ^{13}C NMR lines associated with the $C\beta$ and $C\alpha$ carbons of cysteines binding the cluster which are essentially due to hyperfine interactions between these nuclei and the unpaired electrons present on the cluster. These hyperfine interactions are primarily determined by the characteristics of the magnetic ground state of the active site cluster but also, to a very significant degree, by supplementary contributions coming from their first magnetic excited states populated at the temperature of the experiments (around 300 K).

In the particular case of the $[Fe_4S_4]^{2+}$ state, the excited states with $S = 1$, $S = 2$ ... only contribute to these paramagnetic shifts, since its ground state is diamagnetic ($S = 0$).

In order to investigate the contribution of these excited states, we have undertaken Solid-State ^{13}C NMR experiments using Magic Angle Rotation in two synthetic model compounds : the ^{13}C enriched $[NEt_4]_2 [Fe_4S_4 (SCH_2C_6 H_5)_4]$ compound, and the $[NEt_4]_2 [Fe_4S_4(St Bu)_4]$ compound. These experiments have been performed within a rather large range of temperatures, i.e. between 150 K and 350 K.

It has been possible with this method and with the help of a particular 2D sequence to measure as a function of temperature both the isotropic shifts (essentially due to the Fermi contact hyperfine interactions) and the corresponding anisotropic hyperfine interactions (giving rise to a large collection of rotational side bands). In conjunction with model calculations these measured parameters can then be used to get information on the energy separations between the ground state and the first excited states, and also on the evolution with temperature of the spin populations at the level of the iron atoms.

BIOLOGICAL ACTIVE PALLADIUM(II) HALIDE COMPLEXES WITH DIALKYL α-ANILINOBENZYLPHOSPHONATES

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As a part of our continuing studies on palladium(II) halide complexes with various aminophosphonic acid derivatives which have been found effective in the treatment of some animal and human carcinomas [1,2], new palladium(II) chloride and palladium(II) bromide complexes with diethyl and dibutyl esters of α-anilinobenzylphosphonic acid and [α-(4-benzeneazoanilino)-N-benzyl]phosphonic acid, have been prepared and studied.

Two types of palladium halide complexes, the adducts and the cyclometallated derivatives, have been obtained. Dihalide complexes with 1:2 metal-to-ligand ratio, $Pd(L)_2X_2$ (L = organophosphorus ligand, X = Cl, Br), contain N-coordinated ligand in a *trans* square-planar fashion bonded to palladium through the anilinobenzyl nitrogen in α-anilinobenzyl-phosphonate complexes and through the azo nitrogen in [α-(4-benzeneazoanilino)-N-benzyl] phosphonate complexes, respectively. Azobenzene containing ligands by *ortho*-metallation also form dichloride binuclear palladium complexes, $[Pd(L-H)Cl]_2$, with the metal-metal chloro bridge.

A detailed infrared, electronic and nuclear magnetic resonance spectroscopic studies, accompanied by the FAB mass spectrometric analysis, as well as thermogravimetric, magnetic and conductometric measurements, have been applied for identification and characterization of these complexes with potential pharmacological interest.

1. Lj. Tušek-Božić, I. Matijašić, G. Bocelli, G. Calestani, A. Furlani, V. Scarcia and A. Papaioannou, J. Chem. Soc., Dalton Trans., (1991) 195.

2. Lj. Tušek-Božić, I. Matijašić, G. Bocelli, P. Sgarabotto, A. Furlani, V. Scarcia and A. Papaioannou, Inorg. Chim. Acta, 185 (1991) 229.

A THEORETICAL STUDY OF THE FeMo-COFACTOR

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Abstract

The electronic structure of $[Mo_m Fe_n S_k O_l N_t]^q$ (where $-2 <= q <= 2$; $m=0-6$; $n=0-7$; $k=0-8$; $l=0-4$ and $t=2$) modelling the centre of the FeMo-cofactor has been studied . The calculations are performed for complex , various formal oxidation states of central atoms. The changes of orbitals energies studied as a function of structure and the ratio of atoms in complexes. The results have been applied for the interpretation the redox properties. The cluster $Fe_6 MoS_6^{2-}$ has been found as an only complex which has required ratio of atoms . The energy difference between HOMO and LUMO (ΔE_{HL}) agree well agreement with the redox potential.

Chiral Recognition in Aqueous Solution: Enantioselective Formation of Ternary Copper(II) Complexes of (S)-Amino Acid Amides with (R)- or (S)-Amino Acids.

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In a general project [1] aimed at studying the mechanism of chiral recognition, we have studied the ability of Cu(II) complexes of (S)-amino acid amides added to the eluent to perform enantiomeric discrimination of amino acids in HPLC (reversed phase) [2].

In order to establish the role of the thermodynamic enantioselectivity in aqueous solution and the overall chromatographic mechanism, we have investigated the equilibria between Cu(II), (S)-amino acid amides (phenylalaninamide, prolinamide, tryptophanamide) and (R)- or (S)-amino acids by potentiometry at 25 °C and $I = 0.1$ M KCl.

Remarkable enantioselectivity has been found for the systems (S)-tryptophanamide / proline, (S)-prolinamide/tryptophan, and (S)-phenylalaninamide/proline, the diastereomeric complexes with homochiral ligands being more stable than with the heterochiral ligands; the opposite is true for the systems (S)-phenylalaninamide/histidine, (S)-phenylalaninamide/tyrosine, and (S)-tryptophanamide/histidine.

The observed enantioselectivities will be discussed on the basis of repulsive and/or hydrophobic interactions between the ligand side chains. The results allow to draw some conclusions on the chiral discrimination of amino acids in HPLC.

[1] R. Marchelli, A. Dossena, G. Casnati, F. Dallavalle, S. Weinstein,
    Angew. Chem. Int. Ed. **24**, 336 (1985)
[2] G. Galaverna, R. Corradini, E. de Munari, A. Dossena, R. Marchelli,
    J. Chromatogr. **441**, 287 (1993)

Synergism in peroxidase-catalyzed oxidation of substrates

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Abstract

The peroxidase from the inkcap *Coprinus cinereus*, CiP, has attracted much attention lately [1-10], and recently the crystal structure was solved for a recombinant version, rCiP [7-10]. Although there is a high degree of homology between CiP and lignin peroxidase [5-6], and the structures also are similar [9-10], CiP in its substrate specificity and kinetics more closely resembles horseradish peroxidase (HRP). For a range of substrates common to these two peroxidases, however, CiP has considerably higher specific activity.

In the present work, rCiP has been studied with several substrates. One of these is **ABTS**, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)(2-), commonly used in, e.g., peroxidase activity assays and for hydrogen peroxide determination (usually with HRP).
The formation of the ABTS radical and its spectral properties and lifetime have been studied as a function of pH and other conditions.

Furthermore, the *synergism* between rCiP oxidation of ABTS and other substrates has been studied, i.e., ABTS may act as an enhancer or accelerator of these other rCiP-catalyzed oxidations. Data to elucidate the mechanism of this synergy have been generated.

Using ABTS as an enhancer or auxiliary substrate, peroxidase systems may be made to function on an extended timescale in alkaline media where the systems would otherwise undergo rapid inactivation. Systems of this kind, where the 'other' substrate is added gradually over time, have been parametrized using a simple kinetic model to get an impression of the actual lifetime of the systems under the conditions imposed.

References:

[1] Yuhei Morita et al. 1988.
Purification. Crystallization. and Characterization of Peroxidase from Coprinus cinereus.
J. Biochem. 103. 693-699.

[2] Morten B. Andersen. Yuchiong Hsuanyu. Karen G. Welinder. Palle Schneider & Hugh B. Dunford. 1991.
Kinetics and Equilibria of Cyanide Binding to Coprinus cinereus Peroxidase.
Acta Chem. Scand. 45. 206-211.

[3] Morten B. Andersen. Yuchiong Hsuanyu. Karen G. Welinder. Palle Schneider & Hugh B. Dunford. 1991.
Spectral and Kinetic Properties of Oxidized intermediates of Coprinus cinereus Peroxidase.
Acta Chem. Scand. 45. 1080-1086.

[4] Marianne Kjølke. Morten B. Andersen. Palle Schneider. Bjørn Christensen & Karen G. Welinder. 1992.
Comparison of Structure and Activities of Peroxidases from Coprinus cinereus. Coprinus macrorhizus and Arthromyces ramosus.
Biochim. Biophys. Acta 1120. 248-256.

[5] Lone Baunsgaard. Henrik Dalbøge. Gunnar Houen. E. Michael Rasmussen & K.G. Welinder. 1993.
Amino acid sequence of Coprinus macrorhizus peroxidase and cDNA sequence encoding Coprinus cinereus peroxidase.
Eur. J. Biochem. 213. 605-611.

[6] Karen G. Welinder. 1992.
Superfamily of plant. fungal and bacterial peroxidases.
Current Opinion in Structural Biology 2. 388-393.

[7] Jens P. W. Petersen et al. 1993.
Crystallization and X-Ray Diffraction Analysis of Recombinant Coprinus cinereus Peroxidase.
J. Mol. Biol. 232. 989-991.

[8] Naoki Kunishima et al. 1993.
Crystallization and Preliminary X-Ray Diffraction Studies of Peroxidase from a Fungus Arthromyces ramosus.
Proteins: Structure. Function. and Genetics 15. 216-220.

[9] Naoki Kunishima et al. 1994.
Crystal Structure of the Fungal Peroxidase from Arthromyces ramosus at 1.9 Å Resolution.
J. Mol. Biol. 235. 331-344.

[10] Jens P. W. Petersen. Anders Kadziola and Sine Larsen. 1994.
Three-dimensional structure of a recombinant peroxidase from Coprinus cinereus at 2.6 Å resolution.
FEBS Lett. , submitted.

Structure of metal site in
azurin, Met121 mutants of azurin and stellacyanin
A ^{111m}Cd-PAC-study.

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The geometries of the metal sites in cadmium substituted azurins from *Pseudomonas Aeruginosa* have been investigated by ^{111m}Cd- perturbed angular correlation (PAC). The study includes wild type azurin as well as Met121 mutants of azurin, where methionine has been substituted by Gln, Asn, Asp, Ala, Leu and Glu.

The nuclear quadrupole interaction of wild type azurin analyzed in the angular overlap model is well described as coordination of His46, His117 and Cys112 and cannot be described by coordination of neither Met121 nor Gly45.

For most of the mutants there exists two coordination geometries of the cadmium ion. With the exception of the glu and asp mutants one of the conformations is similar to the wild type conformation. The other coordination geometries are either best described by a coordinating water molecule close to the methionine position or by coordination by the substituting amino acid. These experiments show that even though the methionine does not coordinate it plays an important role for the geometry of the metal site.

The metal site of stellacyanin from Japanese lacquer tree has also been investigated. The nuclear quadrupole interaction is close to both of the two rather similar nuclear quadrupole interactions measured for Met121Gln azurin, indicating that the structure of the metal site in stellacyanin is similar to that of Met121Gln azurin.

Saturation Magnetization Studies of Manganese Substituted Non Heme Iron Enzymes

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In the past decade non heme iron enzymes have emerged as an independent class of proteins mostly involved in dioxygen activation. Ribonucleotide reductase (RNR) and methane monooxygenase (MMO) are the most prominent members of this class. RNR is a complex between protein R1 which binds the nucleotide substrate and protein R2 which contains the dimetal site. The latter protein shows a strong similarity to MMO hydroxylase protein which catalyze the oxygenation of organic substrates when associated with an electron carrying protein and a regulatory protein. X-ray diffraction studies have been used in the last four years to determine the structure of RNR dimetal site in the oxidized ($Fe^{III}Fe^{III}$), reduced ($Fe^{II}Fe^{II}$) and manganese-substituted ($Mn^{II}Mn^{II}$) forms. The structures of the latter two forms are very similar, especially in that the two metal ions are bridged by two μ-1,3 carboxylate groups. Very recently the structure of MMO hydroxylase has been determined in its oxidized ($Fe^{III}Fe^{III}$) form. However, structural information is lacking for the reduced MMO hydroxylase. We have used saturation magnetization techniques to investigate the structural similarity of the two $Mn^{II}Mn^{II}$ sites and therefore derive structural information on the reduced MMO hydroxylase.

Structural Studies on Iron(III) Complex Containing (Z)-2-(2-Amino-4-thiazolyl)-N-(2-hydroxyethyl)-2-hydroxyiminoacetamide

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(Z)-2-(2-Amino-4-thiazolyl)-N-(2-hydroxyethyl)-2-hydroxyiminoacetamide (L) (Fig 1) is the 7-side chain of an orally active semisynthetic cephalosporin antibiotic, Cefdinir[1] (Fig. 1). The structure of L is characterized by oxime, amide and thiazol groups, which obviously have potential for coordination to metal ions. Bioavailability of Cefdinir is indicated to be decreased by co-administration with iron containing drugs, for which coordination to the iron may be responsible. We have determined the molecular structure of a Fe(III) complex of L by means of X-ray crystallography to clarify interaction between Cefdinir and iron.

The structure contains an trinuclear complex Fe_3L_6 (Fig. 2). The moleculer structure consists of a linear Fe-Fe-Fe arrangement, the central Fe atom being an inversion center. Each Fe atom of outside is coordinated to three molecles of L through thiazol N and deprotonated oxime N. Each of the two complex units, FeL_3 is related to an identity, and is adopted by the central Fe atom, which is coordineted to six deprotonated oxime O atoms. We will present results of Mösbauer and ^1H NMR studies (Evans method) on the valences of the three Fe atoms of the complex.
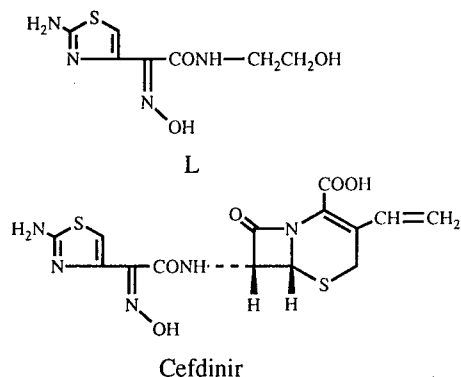
Fig. 1 Structures of Cefdinir and L
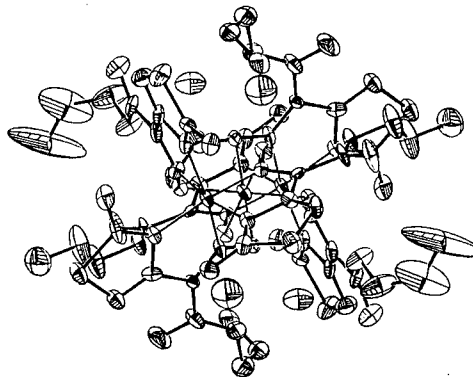
Fig. 2 Molecular Structure of Fe_3L_6

1) T. Nishino, K. Hatano and E. Iwao, *Chemotherapy*, 37(S-2), 77 (1989).

Mode of binding of carboxylate anions at the non-heme iron of Photosystem II in competition with bicarbonate.

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Bicarbonate is the physiological ligand of the non-heme iron of the iron quinone complex of Photosystem II (PS II). Removal of this molecule limits severely the electron transfer in PS II. Despite its significance, the mode of binding and function of bicarbonate is not clear. In a recent study [1,2] we showed that a number of exogenous carboxylate anions (glyoxylate, glycolate, pyruvate, oxalate etc.) can bind on the iron in competition with bicarbonate. We investigate here the effects of these anions on the crystal field axes of the iron octahedron.

We have studied the orientation dependence of the EPR signals of the oxidised form of the non-heme iron, Fe^{3+}, in oriented thylakoid membranes and the more characteristic results are the following : 1) In the presence of glycolate, glyoxylate or pyruvate the Fe^{3+} EPR signals at $g_x \sim 8$ and $g_z \sim 5$, have different orientation in comparison to the intact system [3]. In the case of glyoxylate and glycolate the g_x axis is almost 75-80° with respect to the thylakoid membrane plane (parallel when bicarbonate is bound). In all cases the g_z axis lies parallel to the membrane plane. 2) The tranferin-like Fe^{3+} EPR signal at g 4.3, which is induced at pH>7 in a fraction of centres, is also orientation dependent. In this case g_x and g_y are parallel to the membrane plane. Possible modes of binding of the carboxylate ligands to the non-heme iron of PS II are discussed.

[1] "Binding of carboxylate anions at the non-heme Fe(II) of PSII. I. Effects on the $Q_A^- Fe^{2+}$ and $Q_A Fe^{3+}$ EPR spectra and the redox properties of the iron". Y. Deligiannakis, V. Petrouleas and B.A. Diner, Biochim. Biophys. Acta, in press.

[2] "Binding of carboxylate anions at the non-heme Fe(II) of PSII. II Competition with bicarbonate and effects on the Q_A/Q_B electron transfer rate" V. Petrouleas, Y. Deligiannakis and B.A. Diner, Biochim. Biophys. Acta, in press.

A THERMODYNAMIC ANALYSIS OF CYTOCHROME C_6 AND PLASTOCYANIN OXIDATION BY PHOTOSYSTEM I FROM THE GREEN ALGA *Monoraphidium braunii*.

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Cytochrome c_6 and plastocyanin from the green alga *Monoraphidium braunii* reduce the photooxidized algal photosystem I (PSI) reaction center chlorophyll (P700) with similar kinetics, as expected from their functional equivalence. The observed $P700^+$ reduction rate constants show a non-linear dependence on metalloprotein concentration, which indicates a (minimal) two-step kinetic mechanism involving complex formation prior to electron transfer. The dependence of the observed rate constants on NaCl concentration suggests that the electrostatic interaction forces between the negatively charged donor proteins and PSI are repulsive at neutral pH and relatively low ionic strength, although attractive dipole-dipole interactions may play a role at higher ionic strengths.

Activation parameters for $P700^+$ reduction by cytochrome c_6 and plastocyanin have been determined by studing the temperature dependence of the respective rate constants at varying ionic strength and pH. Changes in NaCl concentration and pH actually induce significant changes in the activation free energy of the overall reaction, even though the corresponding values for activation enthalpy and entropy undergo changes in opposite directions. Such a compensation effect between enthalpy and entropy is observed with both cytochrome c_6 and plastocyanin. Protein concentration dependence of the observed rate constant at different temperatures has allowed an estimate of the free energy change during complex association, as well as the activation parameters of electron transfer, according to a two-step kinetic model.

METAL-ION INDUCED B→Z TRANSITION OF DOUBLE-STRANDED POLYNUCLEOTIDES: EQUILIBRIA, STOICHIOMETRY, KINETICS

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At low ionic strength conditions, small amounts of several transition metal ions, in particular Ni^{2+}, transform the right-handed double-helical form (B-form) of certain polynucleotides into a left-handed one (Z-form). The B→Z transitions are monitored most favorably by CD measurements but can be observed also by the uv spectral changes. We have studied the equilibria for the binding of Ni^{2+}, Co^{2+} and Mg^{2+} to the B-form of poly (dG-dC)·poly(dG-dC) at various ionic strengths, and the conditions under which the conformational transitions of double-stranded poly(dG-dC), poly(dG-m^{5}dC) and poly(dA-dT) take place.

Applying a metal-ion indicator technique enabled a determination of the amount of Ni^{2+} which is bound to the polynucleotide. Parallel CD measurements indicated that at ionic strength 0.001 M the B→Z transition of poly(dG-dC) begins when 1 Ni^{2+} is bound inner-sphere per 4 base pairs and it is complete when 1 Ni^{2+} is bound inner-sphere per 3 base pairs. In the case of poly(dG-m^{5}dC) the transition occurs already when the amount of bound Ni^{2+} is much lower. A strong conformational change is observed also with poly(dA-dT), but it is still not clear yet whether a true Z-structure is formed.

The (slow) kinetics of the B-Z transition of poly(dG-m^{5}dC) has been studied in detail. The reaction curves can be fitted very well by 3 overlapping exponentials. The longest time constant (ca. 20 min., small amplitude) is practically concentration independent, whereas the two shorter time constants depend markedly on the Ni^{2+} concentration, $\tau_2 = 10\text{-}55$ s and $\tau_3 = 60\text{-}300$ s, at ionic strength 0.1 M. A suitable mechanism is proposed and discussed.

The ligands of Cu_A in nitrous oxide reductase

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Nitrous oxide reductase (N_2OR) of *Pseudomonas stutzeri* contains ca. eight copper ions per dimer forming two types of Cu sites. Two Cu atoms per monomer are organized in a binuclear mixed valence [Cu1.5...1.5Cu] center, Cu_A. The other Cu atoms left forms the Cu_z center, a strongly coupled binuclear site (Farrar et al., 1991; Antholine et al., 1992). We have established a system for homologous expression of N_2OR to identify putative ligands of both centers by site-directed mutagenesis. By means of recombinatorial mutagenesis an internal 1555 bp *Sal*I-*Pvu*I fragment of the N_2OR structural gene *nosZ* was replaced by a kanamycin resistance-conferring cassette to generate a *nosZ* null mutant. This mutant can be complemented by a 3500 bp *nosZ*-containing *Xho*I-*Sma*I fragment on plasmid pSUP104. The system was used for PCR-mediated mutagenesis and expression of N_2OR derivatives. The Cu_A-binding motif was identified as a C-terminal region structurally conserved among NosZ proteins from different sources and the subunit II of cytochrome *c*-oxidases (Viebrock and Zumft, 1988; Scott et al., 1989; Zumft et al., 1992). The amino acids D-580, H-583, C-618, C-622, H-626 and M-629 (numbering refers to the unprocessed NosZ protein) were replaced by structurally similar or evolutionary conservative amino acids. These exchanges led to the loss of catalytic activity of N_2OR, tested as whole-cell activity. The mutated residues have, thus, coordinating properties in the Cu_A center, or are required for proper folding and protein stability. The Cu_A-binding site is necessary for the integrity of N_2OR. A mutant deleted in 20 residues preceeding the C-terminal A-638 or the exchange M629D gave no immunochemically detectable protein. Cys→Val exchanges severely impaired the protein export and processing of the signal sequence of N_2OR.

Antholine, W. E., Kastrau, D. H. W., Steffens, G. C. M., Buse, G., Zumft, W. G., Kroneck, P. M. H., 1992. Eur. J. Biochem. 209: 875-881.

Farrar, J. A., Thomson, A. J., Cheesman, M. R., Dooley, D. M., and Zumft, W. G., 1991. FEBS Letts. 294: 11-15.

Scott, R. A., Zumft, W. G., Coyle, C. L., and Dooley, D. M., 1989. Proc. Natl. Acad. Sci. USA 86: 4082-4086.

Viebrock, A. and Zumft, W. G., 1988. J. Bacteriol. 170: 4658-4668.

Zumft, W. G. Dreusch, A. Löchelt, S. Cuypers, H., Friedrich, B., and Schneider B., 1992. Eur. J. Biochem. 208: 31- 40.

ALDEHYDE OXIDO-REDUCTASE ACTIVITY IN *D. desulfuricans* CHARACTERIZATION OF A MOLYBDO-[IRON-SULFUR] ENZYME

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Aldehyde oxido-reductase activity has been found in different sulfate reducing organisms.[1]

The enzyme was now purified to homogeneity from extracts of *D. desulfuricans* ATCC

27774, a sulfate reducer that can use sulfate or nitrate as terminal respiratory substrates.

The enzyme is a molybdo-pterin containing protein, with two additional [2Fe-2S] centers,

and a molecular mass around 200 kDa (homodimer) lacking a flavin group. Visible and EPR

spectroscopies indicate a close similarity with the enzyme purified from *D. gigas*.[2,3]

Activity and substrate specificity towards a wide range of aldehydes was determined (K_M

and V_{max} analysis). EPR spectroscopic studies were extended to aldehyde reacted states of

the enzyme. The significance of this activity, detected in different sulfate reducers, is

discussed in the context of the metabolic pathways used by this bacterial group.

[1]J.J.G.Moura and B.A S.Barata (1994) in "Methods in Enzymology - in press
[2]B..S.Barata, J.Liang, I.Moura, J.LeGall, J.J.G.Moura and B.H.Huynh (1992) *Eur.J.Biochem.* **204**, 773-778
[3]B.A.S.Barata, J.LeGall and J.J.G.Moura (1993) *Biochemistry* **32**, 11559-11568

SYNTHESIS AND STRUCTURE OF
METAL COMPLEXES OF ACYCLOVIR

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Acyclovir (9-[(2-hydroxyethoxy)methyl]guanine, acycloguanosine) is a nucleoside analogue with potent antiviral activity. Acyclovir-triphosphate, being preferentially formed in infected cells, selectively inhibits viral DNA polymerases as compared to cellular polymerases [1]. Some DNA polymerases contain Zn^{2+} and/or are activated by Mg^{2+}, Mn^{2+} or Co^{2+}. Therefore, and in view of the fact, that metal complexes of the drug acyclovir may exhibit antiviral activity different from those of the free ligand, the metal-coordinating properties of acyclovir are of current interest. As part of a study of the coordination properties of purine derivatives [2], we have characterized the following complexes:
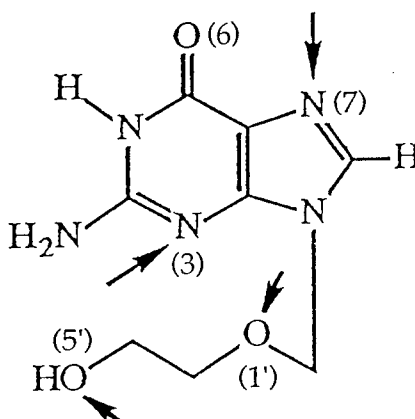
Cu^{II}(acyclovir)Cl_2: $P2_1/n$, V=1201.8 $\mathring{A}^3$, Z=4, R=0.034. Polymeric chain structure; acyclovir bridging two Cu^{2+} ions *via* N(7) (2.01 $\mathring{A}$) and N(3) (2.05 $\mathring{A}$). Additional coordination through O(1') (2.37 $\mathring{A}$) and very weak chelating interaction with O(6) (3.06 $\mathring{A}$) of the purine moiety.

Cd^{II}(acyclovir)$Cl_2 \cdot H_2O$: $P\bar{1}$, V=687.1 $\mathring{A}^3$, Z=2, R=0.028. Chains of chloro-bridged Cd^{2+} ions. Acyclovir bridges these chains by coordinating *via* N(7) (2.41 $\mathring{A}$) and *via* O(5') (2.31 $\mathring{A}$).

Cu^{II}(acyclovir)$(NO_3)_2 \cdot 4H_2O$: $P\bar{1}$, V=884.1 $\mathring{A}^3$, Z=2, R=0.032. Polymeric structure; acyclovir bridging two Cu^{2+} ions *via* N(7) (2.02 $\mathring{A}$) and O(5') (2.24 $\mathring{A}$).

Co^{II}(acyclovir)$(NO_3)_2 \cdot 4H_2O$: $P\bar{1}$, V=882.3 $\mathring{A}^3$, Z=2, R=0.043. Isostructural with the analogous copper complex.

[1] G.B. Elion, Angew. Chem. 101, 893 (1989).   [2] E. Dubler et al., Inorg. Chem. 27, 3131 (1988), 29, 2518 (1990), 31, 3728 (1992), 32, 6095 (1993).

METAL IONS IN ANAEROBIC SLUDGE DIGESTION

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Ions of metals found to be in high concentrations in wastewater of various industries, in that of chemical or metallurgy industries. Heavy metals as Cu,Zn,Pb and Fe accumulate in primary and secondary sewage sludge of industrial andmunicipal treatment plants and can cause damage to thebacteria of anaerobic treatment. It is shown that ions of the heavy metals influence on the process of methane generating and transformating COD,SV,ST,coli fecal, streptococci fecal during anaerobic digestion.

The kinetics of gas generating with different concentrations of Cu (0 - 150mg/l); Fe (0 - 800mg/l); Zn (0-300mg/l); Pb (0 - 300mg/l) was investigated. In order to provide this it was used the installation in batch vohich contained four thermostat reactors ($T=33^{o}C$). An advantage of the installation is possibility of research of different metal concentrations simultaneously. The equation describing dependence ofparameter change rate from metal ions concentration is offered:

$$W = Wo (1 - \alpha[Me]),$$

where Wo is the rate of process with absence of metal ions, $\alpha-$ inhibition constant of anaerobic digestion. It turned out that cuprum ions have a maximum negative influence on anaerobic sludge digestion. According to the degree of the inhibition the metal ions are arranged in the following row:

$$Cu > Zn > Fe > Pb$$

The obtained results way be used to evaluate the effectiveness of the anaerobe process which take place in methane tanks in the presence of compounds of ions heavy metals.

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Theoretical study of models of oxyhemocyanin / oxytyrosinase active sites: Possible link between geometrical arrangements and functional differences.

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Ab Initio calculations on models of oxyhemocyanin and oxytyrosinase active sites indicate that for copper cations coordinated to nitrogens (1 to 3 ligands L around each), the most favorable, energy wize, binding of O_2 occurs with the O-O bond perpendicular to the Cu-Cu direction as in the $(Cu^+)_2$-O_2 naked system[1] and in the experimentally studied complexes.[2] An increase of the Cu-Cu distance is found to be associated, in all cases, to parallel binding of dioxygen.

The study of electronic charactristics of these models for different geometrical arrangements of the ligands L reported in the literature[3] suggests that the relative oritentation of the CuLn groups could be a factor for the functional difference of these enzymes. Thus eclipsed conformations appear, on the basis of electrostatic poetential calculations,[4] to be more reactive than the staggered ones observed in native hemocyanins.[5]

1.   Maddaluno, J.; Giessner-Prettre, C. *Inorg. Chem.* **1991**, *30*, 3439.

2.   a) Jacobson, R. R.; Tyeklar, Z.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *J. Am. Chem. Soc.* **1988**, *110*, 3690. b) Kitajima, N.; Koda, T.; Hashimoto, S.; Kitagawa, T.; Moro-oka, Y. *J. Chem. Soc. Chem. Comm.* **1988**, 151. c) Kitajima, N.; Moro-oka, Y. *J. Chem. Soc. Dalton Trans.* **1993**, 2665.

3.   a) Casella, L.; Gulloti, M.; Bartosek, M.; Pallanza, G.; Laurenti, E. *J. Chem. Soc., Chem. Com.* **1991**, 1235. b) Casella, L.; Gullotti, M.; Pallanza, G.; Laurenti, E. *J. Chem. Soc., Chem. Com.* **1991**, 1611. c) Sanyal, I.; Karlin, K. D.; Strange, W.; Blackburn, N. J. *J. Am. Chem. Soc.* **1993**, *115*, 1259. d) Begley, M. J.; Hubberstey, P.; Walton, P. H. *J. Chem. Soc. Chem. Com.* **1989**, 502.

4.   Eisenstein, O.; Giessner-Prettre, C.; Maddaluno, J.; Stussi, D.; Weber, J. *Arch. Biochem. Biophys.* **1992**, *296*, 247.

5.   a) Volbeda, A.; Hol, W. G. J. *J. Mol. Biol.* **1989**, *209*, 249. b) Hazes, B.; Magnus, K. A.; Bonventura, C.; Bonaventura, J.; Dauter, Z.; Kalk, K. H.; Hol, W. G. J. *Protein Science* **1993**, *2*, 597

THE MECHANISM OF NITRILOTRIACETOIRON(III) TRANSFERRIN INTERACTION.

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Abstract. The role of the protonation of the transferrin aminoacid ligands involved in complex formation and dissociation with iron in the presence of nitrilotriacetate is elucidated. The C-terminal site of transferrin binds to Fe(NTA) to produce a first mixed complex; second order rate constant $k_1 = (7.00 \pm 0.05) \times 10^3$ mol^{-1} dm^3 s^{-1} and a stability constant K_1 $(1.00 \pm 0.10) \times 10^{-5}$ mol dm^{-3}. This lowers the deprotonation pK_a of probably the phenolic side chain of one tyrosine which loses a proton; dissociation constant $K_{1a} = (4.50 \pm 0.50) \times 10^{-7}$ mol dm^{-3} and a possible complex stability constant $K'_1 \approx 2.3 \times 10^{-9}$ mol dm^{-3}. As for the N-terminal site, it binds to Fe(NTA) by a process controlled by a slow proton-transfer; second-order rate constant $k_2 = (4.50 \pm 0.30) \times 10^6$ mol^{-1} dm^3 s^{-1}, a reverse rate constant $k_{-2} = (0.40 \pm 0.05)$ s^{-1} and a proton dissociation constant $K_{2a} = (8.5 \pm 1.1) \times 10^{-8}$ mol dm^{-3}. Fe(nta) release from the N-terminal site of a transferrin both sites of which are loaded with Fe(nta) is controlled by the slow protonation of the mixed protein-Fe(nta) complex; second order rate constant $k_3 = (9.95 \pm 0.35) \times 10^4$ dm^3 mol^{-1} s^{-1}. Protonation of a protein ligan in the C-terminal site leads to a new protein site C loaded mixed complex; stability constant $K_4 = (3.00 \pm 0.25) \times 10^{-4}$ mol dm^{-3}. Fe(nta) release is the result of the mixed complex dissociation and the slow rate-limiting protonation of another protein ligand of the site; proton dissociation constant $K_{5a} = (1.00 \pm 0.10) \times 10^{-4}$ mol dm^{-3} and a second order rate-constant $k_5 = (4.20 \pm 0.40) \times 10^3$ dm^3 mol^{-1} s^{-1}. The mechanism of iron uptake and release indicates that slow proton transfers are the main triggers for complex formation between iron and the aminoacid ligands of each of the protein binding sites.

& Deceased.

The Interactions of Iron with Transferrin

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Summary. The role of the proton dissociation of the transferrin aminoacid ligands involved in complex formation with nitrilotriacetoiron(III), Fe(nta), complex in neutral media and in iron loss in acidic media are elucidated. The C-terminal site of transferrin binds to Fe(nta) to produce a first Fe(nta) mixed complex; second order rate constant $k_1 = (7.00 \pm 0.05) \times 10^3$ mol^{-1} dm^3 s^{-1} and a stability constant K_1 $(1.00 \pm 0.10) \times 10^{-5}$ mol dm^{-3}. This lowers the deprotonation pK_a of probably the phenolic side chain of one tyrosine which loses a proton and, thereby, leads to a second mixed complex; dissociation constant $K_{1a} = (4.50 \pm 0.50) \times 10^{-7}$ mol dm^{-3} and a possible complex stability constant $K'_1 \_ 2.3 \times 10^{-9}$ mol dm^{-3}. As for the N-terminal site, it binds to Fe(nta) by a process controlled by a slow proton-transfer; second-order rate constant $k_2 = (4.50 \pm 0.30) \times 10^6$ mol^{-1} dm^3 s^{-1}, a reverse rate constant $k_{-2} = (0.40 \pm 0.05)$ s^{-1} and a proton dissociation constant $K_{2a} = (8.5 \pm 1.1) \times 10^{-8}$ mol dm^{-3}. In acidic media the Fe(nta) release from the N-terminal site of a transferrin both sites of which are loaded with Fe(nta) is controlled by the slow protonation of the mixed protein-Fe(nta) complex; second order rate constant $k_{3a} = (9.95 \pm 0.35) \times 10^4$ dm^3 mol^{-1} s^{-1}. Protonation of the imidazole of the histidine in the C-terminal site leads to a new protein site C loaded mixed complex; stability constant $K_4 = (3.00 \pm 0.25) \times 10^{-4}$ mol dm^{-3}. Fe(nta) release is the result of the mixed complex dissociation and the slow rate-limiting protonation of the carboxylate of aspartate of site C; proton dissociation constant $K_{5a} = (1.00 \pm 0.10) \times 10^{-4}$ mol dm^{-3} and a second order rate-constant $k_{5a} = (4.20 \pm 0.40) \times 10^3$ dm^3 mol^{-1} s^{-1}. These results permitted to investigate iron release from the holtransferrin in mildly acidic and acidic media in the presence of formate, acetate and citrate. It occurs first from the N-site of the holoprotein. It is independent of the nature or the concentration of competing ligands and is controlled by a slow proton-transfer. It is followed by iron loss from the C-site which is initiated by a proton triggered decarbonation of the binding site. The mechanism of iron uptake and release indicates that slow proton transfers are the main triggers for complex formation between iron and the aminoacid ligands of each of the protein binding sites and proton-assisted decarbonation is prior to any iron loss in acidic media. It remains to be shown whether these slow proton transfer controls a change of the conformation of the binding sites or if they occur because of the particular conformations of the binding sites in neutral media. Although performed *in vitro*, these results can explain the important role played by proton-transfers in iron depletion from the protein in acidic endosomes.

Diffusion along DNA - A Possible Step in the Mechanism for Interactions between Anticancer Drugs and DNA

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The detailed mechanism for interactions between inorganic anticancer drugs and DNA, their commonly accepted target in cancer cells, is presently not well understood. Recent kinetic studies of model systems indicate, however, that the rate of specific adduct formation is dramatically influenced by the length of the target oligonucleotide.[1] The postulated mechanism involves pre-association of a cationic aqua complex with the oligonucleotide followed by diffusion along the DNA to the target site.[1] The present work extends this mechanistic interpretation by variations of pH, ionic strength and the platination reagent. The rate of adduct formation decreases with increasing pH which can be interpreted as an effect of an increasing concentration of the unreactive monohydroxo complex in solution. The kinetically determined pK_a value for cis-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)-Cl(OH$_2$)]$^+$ is equal to ca 6.4. The binding constant for the pre-association step can be estimated to ca 10 M^{-1} by simulation of the kinetics obtained for the reaction between T$_8$p(S)T$_8$ and cis-[Pt(NH$_3$)(NH$_2$C$_6$H$_{11}$)Cl$_2$]. Furthermore, the rate of adduct formation with T$_8$p(S)T$_8$ decreases with increasing ionic strength, k=3.1±0.4 M^{-1}s^{-1} (I=0.09 M) and k= 0.63±0.24 M^{-1}s^{-1} (I=1.0 M), but remains above that of Tp(S)T, k=0.12±0.05 M^{-1}s^{-1} (0.09 M≤ I ≤ 0.30 M). The changes in reactivity and magnitude of the binding constants will be discussed in relation to polyelectrolyte theory.[2]

References
1. Elmroth, S.K.C.; Lippard, S.J. *J. Am. Chem. Soc.* **1994**, in press.
2. Anderson, C.F.; Record, M.T. *Ann. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 432 and references therein.

Speciation in the H⁺-vanadate-adenosine/uridine-imidazole Systems

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The bioinorganic chemistry of vanadium has received an increasing interest in recent years. One of the known biochemical effects of vanadium, is its ability to inhibit or activate several enzymes, e.g. ribonucleases.[1] Therefore, the nucleoside systems are of particular interest. Here the full speciation in the systems H⁺-vanadate(V)-adenosine(Ad)/uridine(Ur)-imidazole(Im) will be presented. The studies have been performed in 0.6M Na(Cl) at 25° C using potentiometry (glass electrode) and ^{51}V NMR spectroscopy (500 MHz). For the H-V-Ad system, two species $(V^-)_2 (Ad)_2$ and $(H^+)_1 (V^-)_2 (Ad)_2$ were established. For the H-V-Ur system, the species $(V^-)_2 (Ur)_2$ and $(H^+)_{-1} (V^-)_2 (Ur)_2$ are formed. In the quarternary system the mixed ligand species $(V^-)_1 (Ad/Ur)_1 (Im)_1$ are formed. The formation constants will be given and the speciation illustrated in distribution diagrams. Also, the species will be identified in the ^{51}V NMR spectra.

1. Chasteen, N. D. *Vanadium in Biological Systems*, Kluwer: Dordrecht, The Netherlands, 1990

Amidate Complexes of cis- and trans-$(NH_3)_2PtCl_2$: Formation, Structure and Solution Studies

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In the last years the so called "platinum blues" have been of considerable interest as second-generation platinum antitumor drugs.[1,2] "Platinum blues" are formed by reaction of cis-$(NH_3)_2PtCl_2$ with various amidate ligands. The first "platinum blue" was prepared by Hoffmann and Bugge in 1908, who treated $Pt(CH_3CN)_2Cl_2$ with silver salts.[3] The original "platinum blue" was suggested to contain deprotonated acetamide, the amidate ligand derived from hydrolysis of CH_3CN. More recently Rochon et al. prepared the acetamidate-bridged complex $(DMSO)ClPt(\mu$-$C_2H_4NO)_2PtCl(DMSO)$ by reaction of $K[Pt(DMSO)Cl_3]$ with CH_3CN.[4] The dimer is discussed to be an analogue of "platinum blue".

Here we report on the hydrolysis reaction of the platinum acetonitrile complex cis-$[(NH_3)_2Pt(CH_3CN)Cl]ClO_4$ (1) leading to the amidate species cis-$(NH_3)_2Pt(NHCOCH_3)Cl$ (2). The starting compound 1 was characterized by X-ray analysis. In aqueous solution 2 is slowly converted to the acetamidate-bridged dimer cis-$[(NH_3)_2Pt(\mu$-$C_2H_4NO)]_2(ClO_4)_2$ (3). Solution studies of 3, which revealed a head-to-head to head-to-tail isomerization equilibrium, are presented.

The reaction of 1 is compared with the hydrolysis of CH_3CN coordinated to trans-$(NH_3)_2Pt(II)$ yielding trans-$(NH_3)_2Pt(NHCOCH_3)Cl$ (4) and the mixed ligand complex trans-$[(NH_3)_2Pt(NHCOCH_3)(CH_3CN)]ClO_4$ (5). The structure of 5 was determined by X-ray analysis.

References:

1. J. P. Davidson, P. J. Faber, R. G. Fischer, S. Mansy, H. J. Peresie, B. Rosenberg, L. VanCamp, Cancer Chemother. Rep. 59, 287 (1975).
2. B. Rosenberg, Cancer Chemother. Rep. 59, 589 (1975).
3. K. A. Hoffmann, G. Bugge, Ber. Dtsch. Chem. Ges. 41, 312 (1908).
4. F. D. Rochon, P. C. Kong, R. Melanson, Inorg. Chem. 29, 1352 (1990).

MÖSSBAUER SPECTROSCOPY OF TRIGONAL-IRON COMPLEXES

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Kim and Rees[1] have proposed a structure for the iron-molybdenum cofactor (FeMoco) of molybdenum nitrogenase. Six of the seven cluster iron atoms are described as trigonal. Only two trigonal iron(II) complexes have been reported[2] with sulfur ligation of the iron atoms. These are the dimeric complexes $[Fe(SAr)_2]_2$ ($Ar = C_6H_2{}^tBu_3$-2,4,6 or $C_6H_2Ph_3$-2,4,6).
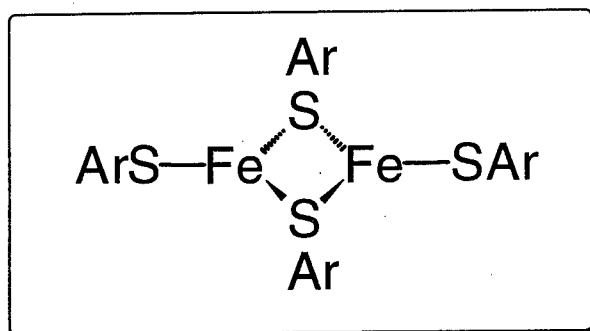
Described here are the Mössbauer parameters for these and three other related complexes ($Ar = C_6H_2Me_3$-2,4,6, $C_6H_2{}^iPr_3$-2,4,6 or C_6F_5). The isomer shifts are consistent with high-spin iron(II) and the quadrupole splittings are unusually large. A comparison is made to the spectrum of FeMoco.

1.   J. Kim and D.C. Rees, *Science*, 1992, **257**, 1677; M.K. Chan, J. Kim and D.C. Rees, *Science*, 1993, **260**, 792.

2.   P.P. Power and S.C. Schoner, *Angew. Chem. Int. Ed. Engl.*, 1991, **30**, 330; *Bull. Soc. Chim. Fr.*, 1992, **129**, 594.

Applications of Molecular Mechanics and Dynamics Methods to the Structure Elucidation of Metal Sites in Biologically Relevant Molecular Systems.

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Recently developed force-fields for Fe(II), Zn(II) and Pt(II) ions in coordination compounds allow to carry out Molecular Mechanics and Dynamics (MM and MD) calculations on large biologically important molecules and polymers with the aim of elucidating the role of the metal center in determining the most peculiar structural features. The new force-fields therefore extend the potentialities of the MM and MD approaches to a full computer simulation which include explicit treatment of the metal coordination site.

Applications of the new force fields to the study of structure-activity relationships will be presented, with particular emphasis on microperoxidase systems, zinc-fingers proteins and synthetic complexes of Pt(II), analogous to the cis-platinum complex, which are models for interaction with DNA bases.

METAL COMPLEXATION OF 1,2-CYCLOMETHYLENE CARBOXYLIC MONOAMIDE HYDROXAMIC AND CARBOXYLIC DERIVATIVES

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Proton and metal complexes formed in aquatic solution with different monoamidic derivatives of cis- and trans-1,2-cyclohexane dicarboxylic acids containing either a carboxylic or hydroxamic acid group in the side cain, have been studied especially by pH-metric and spectrophotometric methods. The metal ions involved in these studies are: Cu^{2+}, Ni^{2+} and Zn^{2+}. The results show that the carboxylate derivatives form much less stable complexes than the hydroxamate ones. Moreover, differences can be found both in acid-base and metal complexation of cis and trans isomers. These findings are in good correlation with the results published recently[1]. Namely, out of the compounds, designed as potential inhibitors of angiotensin converting enzyme (ACE), the hydroxamate derivatives have been found the most effective ones.

The equilibrium models, the most probable bonding mode of species formed with the different type of ligands and assumption for the possible cause of good correlation between stability of the complexes and inhibitory effect of the ligands will be shown on the poster.

[1]L.Turbanti, G.Cerbai, C.Di Bugno, R.Giorgi, G.Garzelli, M.Criscuoli, A.R.Renzetti, A.Subissi and G.Bramanti, J.Med.Chem., 36. 699 (1993)

Low Temperature Magnetic Circular Dichroism Studies
of the Dimeric Cu_A Centre

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The electron transfer centre Cu_A is common to bacterial nitrous oxide reductases and both mammalian and bacterial aa_3 type cytochrome $\underline{c}$ oxidases. The presence of additional chromophores has previously complicated spectroscopic characterisation of this centre. The recent isolation of both a mutant nitrous oxide reductase containing only the Cu_A centre and the expression of subunit II (containing only Cu_A) of the bacterial cytochrome oxidase from *Paracoccus denitrificans* and *Synochocystis* in *E. coli* has allowed the optical properties of this centre to be more fully explored.

This poster presents comparative absorption, EPR and low temperature MCD data from the native Cu_A centre in *Pseudomonas stutzeri* and *Thiosphaera pantotropha* nitrous oxide reductases, *Paracoccus denitrificans* and *Synochocystis* cytochrome $\underline{c}$ oxidases as well as that from a Cu_A centre constructed by site directed mutagenesis of the quinol oxidase, cytochrome bo, of *E. coli*.

Increasing the pH from 6 to 10 has a marked effect on the spectral characteristics of the *P. dentrificans* Cu_A domain, with the intense absorption and MCD bands disappearing and the EPR spectrum losing the 7-line hyperfine splitting pattern. Subsequent site directed mutagenesis of the proposed copper ligand, His252, has resulted in a species which exhibits comparable spectra even at low pH. EPR, optical and MCD spectra of this and other mutants which retained copper are presented here.

A simple model for a binuclear copper A centre is proposed which accounts for the observed spectroscopic features.

Reactions of d^8 Metal Ions (Pd^{II}, Pt^{II}, Au^{III}) with Mono- and Trinucleotides

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Principal binding patterns of metal ions with nucleobases can be studied, as far as coordination to the heterocyclic part is concerned, by use of simple models such as N1-alkylated pyrimidines or N9-alkylated purines.[1] With soft metal ions like Pd^{II}, Pt^{II} and Au^{III}, these binding modes are expected to be also relevant to nucleosides and nucleotides, even though in the latter case phosphate binding is possible as well.

We have tested this hypothesis by studying reactions of $(dien)Pd^{II}$, cis-$(NH_3)_2Pt^{II}$ and $(dien)Au^{III}$ with 5'-dGMP, d(GpTpG) and d(ApTpG).

(1) $(dien)Pd^{II}$ reacts with 5'-dGMP to give N7-, N1,N7-, N1,N7,PO-, N1,N3,N7,PO-adducts in neutral solution. Phosphate binding is observed, together with ring binding, only at $r(Pd^{II} : 5'\text{-dGMP}) > 2$. Phosphate binding is not observed under acidic conditions and is clearly disfavoured as compared to ring binding.

(2) cis-$(NH_3)_2Pt^{II}$ reacts with d(GpTpG) by forming a guanine-N7-, guanine-N7-chelate.[2] Addition of $(dien)Au^{III}$ results in formation of a mixed metal complex with Pt^{II} binding at guanine-N7, guanine-N7 and Au^{III} coordinating at N3 of thymine. This finding confirms the high selectivity of $(dien)Au^{III}$ for N3 of thymine[3] and may provide a useful approach for identification of GTG cross-links of cisplatin in DNA.

(3) Analogous reactions of cis-$(NH_3)_2Pt^{II}$ and $(dien)Au^{III}$ with the trinucleotide d(ApTpG) have been examined.

Lit.:
1. B. Lippert, Prog. Inorg. Chem. **1**, 37 (1989).
2. (a) M. Garner, M. Green, N. Boogaard and J. Reedijk, J. Chem. Soc., Chem. Commun. **3**, 290 (1993).
   (b) N. Boogaard, C. Altona and J. Reedijk, J. Inorg. Biochem. **49**, 129 (1993).
3. B. Fischer and B. Lippert, J. Inorg. Biochem. **43**, 432 (1992).

Studies on the interaction of some designed platinum complexes with DNA

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As an attempt to quantify the nature of interaction of platinum-based anti-cancer drugs with DNA, we have carried out interaction studies for a number of designed platinum complexes. We report here the results of interaction of our desinged platinum complex code named AMBO with 9-ethyladenine, 9-ethylguanine, G, A, GG and AG. The complex has two cis-chloride ligands as in cisplatin, but the ammino ligands have been replaced by a cyclic structure which coordinates with platinum through an amino group and an N-hydroxy group. The reactants - the complex, the base, the mononucleotide or the dinucleotide - were each dissolved in 0.020 mM $NaClO_4$, pH 5.5. The solutions were mixed and incubated for 24 h at 37°C. For the mononucleotides, the complex and and the nucleotide were mixed in the molar ratio of 1:2 whereas for the dinucleotides, the ratio was 1:1. The adducts were separated by HPLC. A HPLC system consisting of two Waters (Milford, MA, USA) 510 pumps, and a 484 Waters absorbance detector, a WISP 712 (Waters) auto-injector and Maxima 820 chromatography workstation were used. Eluent A 0.1 M NH_4Ac, pH 5.5; eluent B MeOH). The column used was a resolved radialpak C_{18} column (8 mm x 100 mm). Platinum content of each HPLC fraction was determined by AAS. A Varian Spectraa-20 AAS with graphite furnace facility was used. The UV absorption at 260 nm was used to determine nucleotide contents of HPLC fractions.

With 9-ethyl adenine 1 : 1 adduct was predominant and 9-ethyl guanine 1 : 2 adduct was predominant. With mononucleotides 1: 2 (Pt : nucleotide) adducts were predominant. For dinucleotides, a number of adducts formed, the predominant ones being 1 : 1 (Pt : dinucleotide) adducts. With AMBO plus AG four isomeric 1 : 1 adducts were formed.

FURTHER INVESTIGATIONS ON THE HIGHLY PURIFIED LACTOPEROXIDASE Fe(III)-HEME CATALYTIC SITE

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Purification of the lactoperoxidase (LPO) major cationic isoenzyme was recently improved in our laboratory, by the use of preparative chromatographic and electrophoretic methods combined with analytical electrophoretic techniques and image processing [1].

The electron paramagnetic resonance has played a fundamental role in evaluating the enzyme purity against lactoferrin and minor LPO isoenzyme components in setting the final steps of the purification.

The LPO1 EPR spectrum, at physiological pH, was clearly indicative of the presence of an iron (III)-heme high spin catalytic site in the native enzyme. The LPO/SCN⁻ activity at optimum pH ($\cong$ 5.5) has been measured in phosphate and acetate buffer.

Spectroscopical results together with activity and kinetic data seemed to indicate the insertion of thiocyanate as ligand bridge between Fe(III)-heme and N_3H imidazole distal histidine to give a small Fe(III) interaction at room temperature which indeed became stronger at low temperature.

With the aim to better clarify the LPO structure and heme catalytic role, we investigated on the partially analyzed bovine milk LPO primary structure, previously reported in the literature [2].

At the same time we pursued our kinetic studies on the LPO/SCN⁻ system in the presence of inorganic and organic inhibitors respectively.

References:
[1] Ferrari R.P., Laurenti E., Cecchini P.I., Gambino O., Sondergaard I., submitted for publication (1994)
[2] Cals M.-M., Mailliart P., Brignon G., Anglade P. and Dumas B.R. J. Biochem. 198, 733 (1991)

THE BENEFITS OF GLOBAL ANALYSIS WITH NUMERICAL INTEGRATION APPLIED TO MULTIVARIATE SPECTROSCOPIC DATA FOR THE ELUCIDATION OF KINETIC MECHANISMS AND INTERMEDIATE SPECTRA

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The use of stopped-flow absorption and fluorescence spectroscopy to determine reaction mechanisms and rate constants in biochemical systems has long been established. Traditional non-linear least squares analysis of a kinetic trace is routinely used to determine the number and magnitude of exponential components in the reaction. More recently the introduction of instruments which generate high quality kinetic data at multiple wavelengths have given the kineticist the potential to establish these reaction parameters with greater precision and the opportunity to analyse reactions of far greater complexity. However the size of the data matrix is great, requiring a large amount of computer memory for storage and placing much more emphasis on data handling and analysis.

We demonstrate the use of **Single Value Decomposition** as a means of reducing the size of data sets as well as providing a model free assessment of the minimum complexity of the reaction mechanism prior to analysis. And on the use of **global analysis with numerical integration** to determine rate constants and intermediate spectra based upon virtually any proposed reaction mechanism.

From RNA to DNA : a "radical" evolution.
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Reduction of ribonucleotides to deoxyribonucleotides is a key chemical event of life, within the DNA world. While such a reaction still remains an intriguing challenge for organic chemists, all living organisms have evolved active ribonucleotide reductases which provide DNA precursors in a tightly regulated way. So far, three classes of ribonucleotide reductases have been isolated and characterized. Class I enzymes are found in aerobes such as bacteria (*Escherichia coli*) bacteriophages (T4), viruses (herpès simplex) and mammals. Enzymatic activity depends on a tyrosyl radical and a binuclear non heme iron site. Class II enzymes are found in prokaryotes, anaerobes or aerobes, a prototype of which was extensively studied in *Lactobacilus Leichmanii*. Activity depends also on a metal cofactor residing in the cobalt ion of vitamin B12. Class III enzymes were recently discovered in anaerobes. They are rapidly inactived by oxygen and contain an iron-sulfur cluster and a glycyl radical, both alsolutely required for activity. It thus appears that all ribonucleotide reductases require a metal cofactor and proceed by radical mechanisms. The structure and the function of the different metallic sites will be discussed in order to show that they have in common to participate to the generation and the stabilization of reactive free radicals, including protein radicals. Those radicals are then utilized to activate the ribose moiety of the ribonucleotide substrate, thus allowing its reduction by redox-active cysteines of the polypeptide chains. In order to understand the evolution of ribonucleotide reductases, it is important to delineate the similarities as well as the differences between the various classes, in terms of oxygen sensitivity, structure complexity, allosteric regulation, structure of the metal cofactor, radical mechanisms. From that comparison it is quite convincing that class III enzyme is a primitive ribonucleotide reductase, responsible for the advent of the DNA world from the RNA world, before oxygen appears in the atmosphere.

PRELIMINARY STUDIES ON THE RECENTLY DISCOVERED [2Fe-2S] CLUSTER OF FERROCHELATASE

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Ferrochelatase (protoheme ferrolyase, EC 4.99.1.1) is the terminal enzyme of the heme biosynthetic pathway. Natural ferrochelatase, purified from mouse livers and recombinant ferrochelatase, purified from an overproducing strain of *E. coli* [1], were investigated by EPR and Mössbauer techniques [2]. In their reduced forms, both the natural and the recombinant ferrochelatases exhibited an identical EPR signal with *g*-values 2.00, 1.93 and 1.90 and relaxation properties typical of a $[2Fe-2S]^{+1}$ cluster. Recently, the same type of center was proposed for recombinant human ferrochelatase using EPR and MCD spectroscopies [3]. Mössbauer spectra of the recombinant ferrochelatase, purified from a strain of *E. coli* cells harbouring a ferrochelatase expression plasmid and grown in ^{57}Fe-enriched medium, demonstrated unambiguously the presence of a [2Fe-2S] cluster. Studies are underway to elucidate the putative Fe-S binding site and the possible role of this cluster on the ferrochelatase mechanism and/or activity regulation. This studies may provide insights into the diversified roles of iron-sulphur cores in an expanding number of proteins.

[1] Ferreira, G.C., *J. Biol. Chem.* (1993) **269**, 4396-4400

[2] Ferreira, G.C., Franco, R., Lloyd, S.G., Pereira, A.S., Moura, I., Moura, J.J.G. and Huynh, B.H., *J. Biol. Chem.* (1994) **269**, 7062-7065

[3] Dailey, H.A., Finnegan, M.G. and Johnson, M.K., *Biochemistry* (1994) **33**, 403-407

METAL VERSUS LIGAND OXIDATION IN A BISIMIDAZOLE MnIII, MnIV MODEL COMPLEX FOR THE MANGANESE SITE IN PHOTOSYNTHESIS.

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A $[Mn_2^{III,IV}O_2(bisim)_2]^{3+}$ dinuclear system (denoted [34], where bisim symbolizes N,N'-dimethyl-N,N'-bis[(imidazole-4-yl) methyl] ethane 1,2-diamine) has been synthesized with the following bisimidazole ligand:
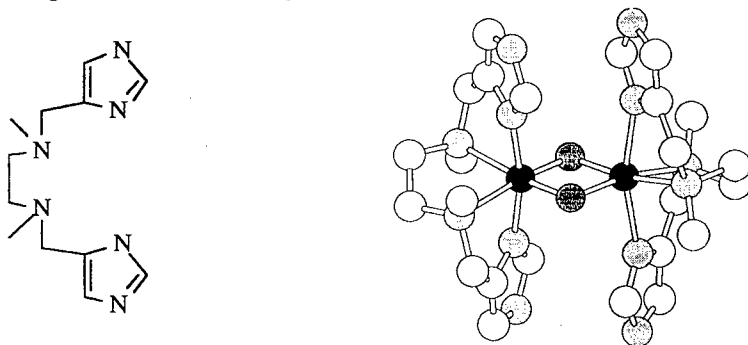
The structure has been characterized. The coordination around MnIII is elongated and the MnIV site is geometrically distinct.

Two reversible oxidation waves are observed at $E°(ECS)=$ 0,8 V, $E°(ECS)=1,2$ V. Coulommetry shows that the first wave corresponds to one electron and the second wave to half an electron per two manganese atoms. E.P.R. spectra recorded for the different species allow us to propose an interpretation of the electrochemical properties which is shown in the following scheme:

$$2[34] \xrightarrow{-2\ e^-} 2[44] \rightleftharpoons [4444] \xrightarrow{-1\ e^-} [4445]$$
$$\text{dimer} \qquad \text{dimer} \qquad \text{tetramer} \qquad \text{tetramer}$$
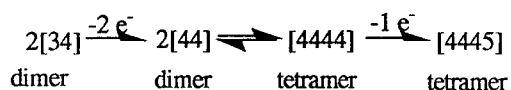
Species [34] exhibits the usual 16 line spectrum, [4444] is E.P.R. silent and the [4445] exhibits a 6 line spectrum. The observation of a 6 line spectrum for the [4445] species can be understood on the basis of the following structural hypothesis:
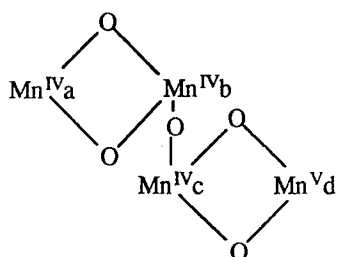
where Mn^V and Mn^{IV} would be respectively S=0 and S=3/2, and Mn^{IV} b and c strongly antiferromagnetic coupled.

U.V. irradiation of the original [34] complex at 77 K in aqueous borate solution allows us to observe by E.P.R. the formation of an imidazole radical weakly coupled to the $Mn^{III}Mn^{IV}$ pair very similar to the signal observed in Ca^{2+} depleted PSII preparations in the S_3 state [ref].

The $[Mn_2^{III,IV}O_2(bisim)_2]^{3+}$ system seems to be a very usefull model for understanding the factors governing metal versus ligand centered oxidation.

ref : A. BOUSSAC & al, Nature, Vol 347, 20 September 1990, P 303-306

Aluminium uptake studies on Escherichia coli under physiological pH and iron limited conditions

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Aluminium is widely regarded as toxic under pH-conditions established in soil and open water under the influence of acidic rain[1].
Previous studies have shown inhibition of growth of Escherichia coli by aluminium nitrate is pH-dependent. The increase of pH from 5.4 to 6.8 decreased the sensitivity to 0.9mM or 2.25mM aluminium whereas omission of iron enhanced aluminium toxicity. The last indicates that a Fe(III) transport pathway may be used for aluminium transport to intracellular binding sites[2].
The work presented in this poster is a study on aluminium uptake in Escherichia coli under iron limited conditions and physiological pH. It shows that under physiological pH aluminium concentrations of up to 2mM do not show growth inhibition under iron sufficient conditions, but under omission of iron the growth pattern changes remarkably indicating a stronger sensitivity towards aluminium concentration.
Furthermore the investigation of aluminium speciation indicates that the composition of the medium in respect of potential aluminium chelating ligands highly influences aluminium toxicity in bacteria.
Aluminium concentrations are also shown to have influence on siderophore production suggesting interference of aluminium with this iron uptake pathway.

1.)B.O.Rosseland, T.D.Eldhuset and M.Staurnes, Environ.Geochem.Health, 1990, 12 1/2,17-27
2.) L.Guida, Z.Saidi, M.N.Hughes and R.K.Poole, Arch.Microbiol.1991,156,507-512

PHYSIOLOGICAL ACTIVITY OF PYRUVYLAMINO ACID OXIMES AND THEIR ANIONIC COMPLEXES WITH 3d-METALS

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Hydroxyimino analogues of dipeptides - pyruvylamino acid oximes (H_3pao) CH3-C(=NOH)-C(O)NH-CH(R)-COOH have been widely investigated during last five years in connection with their strong binding activity towards transition metal ions and because of the marked bioactivity of both ligands and metal complexes. The derivatives of gly, l-ala, l-met, l-phe and d,l-asp were synthesized and investigated both in crystal state and in solution.

A series of pao-containing anionic complexes with cobalt(III), nickel(II) and copper(II) of composition $[Co(pao)_2]^{3-}$ $[Ni(Hpao)_2]^{2-}$, $[Ni(pao)(Hpao)]^{3-}$, $[Cu(pao)]^{-}$ have been obtained, their composition, properties and structure were studied by means of miscellaneous spectral methods and magnetic susceptibility measurements. The crystal structure of four organic substances and two complexes was determined by X-ray analysis.

H_3pao and anionic 3d-metal complexes were tested as substances stimulating nervous system (analeptic action) and affecting system arterial blood pressure. H_3pao are prove to be the substances stimulating cetral nervous system (shortening the barbituric sleeping 1.2 -1.4 times in reference to the control) that close to the activity of the reference substance caffeine. Both the H_3pao and the complexes indicate marked hypotensive activity exceeding that of reference (theobromine) 1.3 - 1.5 and 1.3 - 1.9 times, respectively. The activity of the complexes exceed that of the free ligands 1.1 - 1.5 times and therefore the former is probably to be subject of the further pharmaceutical investigation.

Complex Formation in Aqueous Vanadate Solutions Containing Dipeptides

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Vanadate ($H_2VO_4^-$) binds to the active site of many proteins and, in several cases, histidine (his) is involved. We have modelled this interaction with small peptides such as alanylhistidine (ala-his), prolylalanine (pro-ala) and alanylglycine (ala-gly). Multinuclear NMR spectroscopic results (^{13}C, ^{51}V, ^{1}H) and an equilibrium analysis are presented.

In the case of ala-his the formation of two five-membered rings with vanadate is assumed. The peptide coordinates via the terminal amino and carboxylate groups and the deprotonated amid N. As an example, the complex formation and the proposed structure of ala-his with vanadate is presented in scheme 1 [1,2].
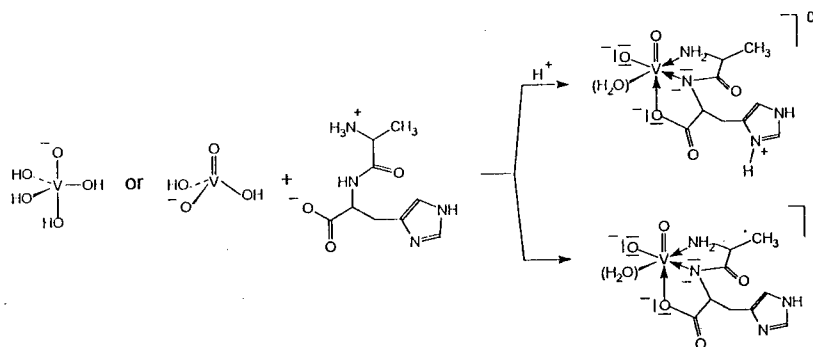
Scheme 1 Formation and proposed structure for the uncharged and -1 charged
         complex

[1] M. Fritzsche, V. Vergopoulos, D. Rehder, *Inorg. Chim. Acta.* **1993**, *211*, 11-16

[2] K. Elvingson, M. Fritzsche, D. Rehder, L. Pettersson, *Acta. Chem. Scand.*
    submitted

Electron Paramagnetic Resonance Studies of Ferric Cytochrome c' from Photosynthetic Bacteria: Unique Electronic Ground States

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Cytochromes c' are c-type heme proteins found in purple photosynthetic, denitrifying, nitrogen-fixing, and sulfur-oxidizing bacteria. Although their physiological function has not been precisely established, it is suggested that they are members of the electron transfer system in the bacterial cell. The electronic ground state nature of the ferric cytochrome c' at physiological pH has been investigated by various spectroscopies. Most investigators suggested the high-spin nature of the heme iron. However, Maltempo and coworkers[1] proposed a quantum mechanical admixed spin state of a high spin ($S=5/2$) and an intermediate spin ($S=3/2$) as the ground state of the ferric cytochrome c' isolated from a photosynthetic bacterium, *Chromatium vinosum*. In the present work, we have been characterized the ground state of the cytochrome c' from five photosynthetic bacteria, *Chromatium vinosum, Rhodobacter capsulatus, Rhodopseudomonas palustris, Rhodospirillum molischianum*, and *Rhodospirillum rubrum*. The unique ground state structure, deduced from EPR spectra, of the cytochrome c' from five bacterial strains will be discussed.

[1] M.M.Maltempo et al., *Biochim.Biophys.Acta*, **1974**, *342*, 290-305

IRON UPTAKE IN BRUSH BORDER MEMBRANE VESICLES:
A COORDINATION CHEMICAL VIEW

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The absorption of iron from the gastrointestinal tract depends on the one hand on the needs of a given animal species, on the other hand on bioavailability of iron in food, i.e. the chemical speciation. However, there are still open questions regarding the mechanisms of the regulation of iron homeostasis as well as the importance of the oxidation state and of the various complexes of iron for its uptake.

The aim of the present work was to assess the potentialities of different iron species for the first step of absorption, the transfer through the brush border membrane. Iron binding to and uptake into rabbit brush border membrane vesicles were determined. The vesicles were incubated in media containing nitrilotriacetate (NTA). With an iron:NTA-ratio of 1:2 iron is partly hydrolyzed and the presence of small polynuclear complexes was indicated. The calculation of the species' concentrations and an assessment of their reactivity and possible interactions with the membrane suggest that the "twins" mononuclear $Fe(OH)_3$ and $Fe(NTA)(OH)^-$ are responsable for transport kinetics. The most remarkable property of $Fe(NTA)(OH)^-$ is its relatively rapid decay into $mn\text{-}Fe(OH)_3$ and $HNTA^{2-}$. Interestingly, the rate of this decay corresponds to the rate of the iron uptake into the vesicles!

Our data support the hypothesis that ferric iron may be efficiently taken up from the gastrointestinal tract if there are ligands which

- provide a sufficient amount of mononuclear iron complexes by catalyzing the release of iron from polynuclear complexes

- form complexes with iron being in a labile equilibrium with $mn\text{-}Fe(OH)_3$.

Computer Modelling of Metalloenzymes

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Metal cations belonging to active sites of enzymes influence activity mainly through electrostatics. Accordingly, we may feel correct to simulate their effect by unpolarizable monopoles bearing +1, +2, or higher, charges. However, there are several cases known where the above assumption fails, charge transfer takes place and an effective fractional charge, less than the formal one, has to be ascribed to the metal. We present two examples where charge transfer plays an important role in enzymatic action. *Cytochrome C peroxidase* (CCP) is a heme protein, it catalyses peroxide reduction and has a Fe^{3+} ion in the centre of the heme. We did open-shell AM1 semiempirical molecular orbital calculations on a model with Be^{3+} replacing the ferric cation and obtained spin densities for a model including the heme and the Arg-48, His-52, His-175, Trp-191 and Asp-235 side chains. Our results indicate that if the Trp-191 residue is protonated on the indole nitrogen, the unpaired electron is located at the heme. On the other hand, if the proton is shifted to Asp-235 the unpaired electron appears at the Trp-191 side chain in agreement with recent experiments. For smaller models containing fewer adjacent side chains the spin density is much less localised. We will compare the effect of various cations on the spin density by applying the ZINDO/1 semiempirical method specifically designed to handle transition metals. *D-Xylose isomerases* catalyse the isomerisation of D-xylose and D-glucose from aldose to ketose and require divalent cations for activation. We examined the charge transfer processes, induced by Be^{2+}, Mg^{2+}, Co^{2+} and Mn^{2+} ions involved in the reaction and attempted to find a relation between activity and charge transfer from the active site to the metal. We found experimentally that the isoenzyme with a Be^{2+} cation is inactive and our calculations indicate that this may be due to the reduced charge transfer to beryllium as compared to other cations.

^{1}H NMR Studies of Oxidized 2[4Fe-4S] Ferredoxins

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The ferredoxins from *Clostridium pasteurianum* (Cp) and *Clostridium acidurici* (Cau) have been investigated by ^{1}H 1D and 2D NMR in their 2[4Fe-4S]$^{2+}$ state. Using conventional TOCSY and NOESY experiments sequential assignments were obtained for the majority of the protons of which the signals are only moderately perturbed by the presence of the paramagnetic iron-sulfur clusters.

For the assignments of the ß-CH$_2$ protons of cysteines which undergo the strongest shifts another strategy was employed. By replacing the conserved prolines at position 19 and 48 or Asp-39, the assignments of cysteines 18, 37 and 40 were inferred from the selective perturbation of their signals in the case of Cp ferredoxin

Our results will be discussed in relation with the crystal structures of 2[4Fe-4S] ferredoxins, particularly concerning the following points: the arrangement of most residues around the clusters, the influence of hydrogen bonds on the main properties of the molecule and the conformation of the crystallographically ill-defined region encompassing residues 25 to 28.

J. Gaillard et al. Biochemistry 32, 9881-9887 (1993)

J. Gaillard et al. Magnetic Resonance in Chemistry 31, S27-S33 (1993)

I. Quinkal et al. Prot. Engng. 7, in press (1994)

Structure and stability of Ni(II) and Cu(II) complexes of histamine-containing oligopeptides

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The complex-formation processes are greatly influenced by the coordination of side-chain donor groups of peptides. In this respect, the imidazole moiety seems to be one of the most important, since it offers a particularly stable metal-binding site in physiological pH range and can be found in a large number of metalloproteins as binding sites of various metal ions. Oligonuclear complexes containing bidentate, bridging imidazole units are also of current interest from biomimic points of view.

In order to get more detailed picture of metal ion coordination of imidazole ring in the peptide chain, we studied the metal coordination properties of some closely related histamine derivates (Gly-HA, Sar-HA and Gly-Gly-HA) by potentiometric and spectroscopic (EPR, NMR) tools, with special attention to oligomerisation processes in alkaline solution.

In case of dipeptides, we have shown that, beside the formation of monomeric affiliated complexes (MLH, ML, MLH_{-1}, ML_2, ML_2H_{-1}, MLH_{-3}), between pH = 9 - 11 oligomeric species $(MLH_{-2})_n$ are also formed which is in equilibrium with the monomeric $MLH_{-1}(OH)$ depending on the concentration. The EPR spectra of diamagnetically diluted solid, oligomeric Cu(II)-complexes entirely compatible with the square-planar structure, and for the first time for such oligomeric species, verified the expected {4N} coordination. The fourth nitrogen donor should be the deprotonated N^1-pyrrolic nitrogen of an another monomeric unit, forming a cyclic oligomer. The variable temperature ^{1}H-NMR and 2D-EXSY measurements of diamagnetic Ni(II) oligomers revealed the formation of only one oligomeric species (probably tetrameric for steric reason) in Ni(II)-Gly-HA system, however showed the coexistence of several oligomers, including monomeric units $n \geq 4$ in Ni(II)-Sar-HA system. The probably explanation of this basic difference is the steric hindrance of $N-CH_3$ group of Sar-hist, which may decrease the salient stability of tetrameric species as compared to the higher homologues.

The tripeptide Gly-Gly-HA may serve as a model for the Cu(II) binding site of serum albumin (similarly as the extensively studied Gly-Gly-His, but latter can rapidly undergo decarboxylation). At physiological pH, the {4N} coordinated $CuLH_{-2}$ complex is the only existing species, reflecting its extremly high stability. The X-ray structure of this complex showed a planar coordination of amino, two amido and N^3-imidazole nitrogen. At higher pH further deprotonation takes place (pK = 11.8) which, on the basis of our NMR and EPR measurements, can be assigned to the deprotonation of N^1-pyrrolic nitrogen of imidazole ring without metal coordination.

Saturation recovery EPR determination of interactions between the tyrosine-radical and the ferric diiron cluster in different R2 subunits of ribonucleotide reductase

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Ribonucleotide reductase (RNR) catalyses the reduction of all four ribonucleotides to their corresponding deoxyribonucleotides, an essential reaction in all living cells. The smaller R2 subunit contains a stable tyrosyl free radical with a 5.3 Å (for the *E. coli* RNR) distance to the closest iron in the µ-oxobridged diiron cluster. The relaxation pathways of the tyrosine-radical of four different RNR R2 proteins have been studied as function of temperature using microwave saturation recovery spectroscopy [1]. At temperatures below 12 K, the bulk magnetization M_z recovers as a single exponential following the excitation pulse with the same values for *E. coli* [1], mouse and herpes simplex virus type 1 (HSV1) R2 proteins. This rate of recovery is the intrinsic spin-lattice relaxation. The *Salmonella* R2, which has a tyrosine-radical similar to the photosystem II tyrosine-radical, shows a lower rate than the other R2 proteins but it is similar to what is observed for the photosystem II tyrosine-radical. For temperatures over 12 K, the recovery rates consist of more than one component, and the temperature dependence of the dipolar and scalar components of the interaction between the radical and the Fe(III)Fe(III) cluster has been analysed. It was possible to obtain an estimation of the exchange coupling constant between the two ferric irons from the temperature dependence of the spin-lattice relaxation data (Table 1). The low value for the Fe(III)Fe(III) exchange coupling for the mouse and the HSV1 R2 proteins might explain why it is possible to make stable mixed valent Fe(II)Fe(III) states of these proteins [2] but not for the *E. coli* R2. The data is consistent with the possibility that the mouse R2 might have an additional weak hydrogen-bond to the oxygen bridging the two ferric ions, which is not present in the *E. coli* R2.

Table 1

	Fe(III)Fe(III)
E. coli	92 cm^{-1}
mouse	45 cm^{-1}
HSV1	62 cm^{-1}
Salmonella	79 cm^{-1}

[1] Hirsh, Beck, Lynch, Que and Brudvig (1992) *J. Am. Chem. Soc.* **114**, 7475-7481

[2] Atta, Andersson, Ingemarson,Thelander and Gräslund (1994) *J. Am. Chem. Soc.*, (submitted)

THE CHEMICAL NATURE OF AMAVADIN

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Mushrooms of the genus *Amanita* accumulate vanadium to a concentration of ≤ 400 mg kg^{-1} (dry weight) in the form of the discrete moiety Amavadin.[1] This is a 1:2 complex of V^{IV} with *S,S*-2,2′-(oxyimino)dipropionate(3-), $^-$ON{CH(Me)CO$_2$$^-$}$_2$ (hidpa^{3-}).[2] The complex may be reversibly oxidized to the V^V level;[3] thus, Amavadin belongs to the group of transition metal centres in biology which exhibit one-electron, reversible redox behaviour. We have established[4] that Amavadin comprises a novel octacoordinated complex, with each hidpa^{3-} ligated *via* the η^2-*N,O* group and two unidentate carboxylate-groups.
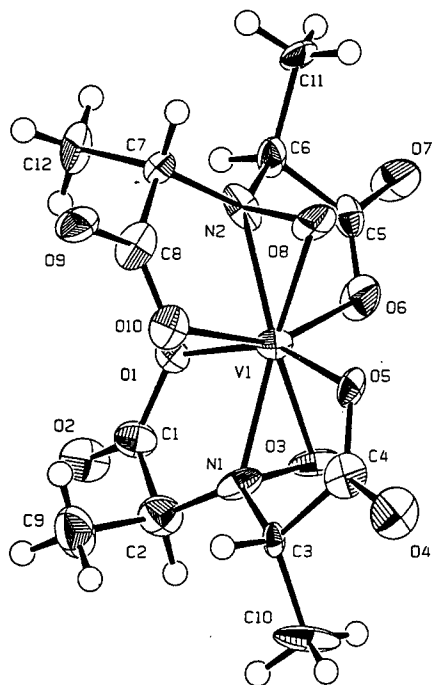
Figure 1. Structure of $[\Lambda$-V(*S,S*-hidpa)$_2$]$^{2-}$ isolated from *Amanita muscaria*.

This coordination environment leads to chirality at the vanadium. Circular Dichroism and NMR spectroscopy have shown[4] that Amavadin, as isolated, consists of an approximately equal mixture of the Δ- and Λ- forms of [V(*S,S*)-hidpa)$_2$]$^{2-}$. We have crystallised the Λ-form of this anion (Figure 1). A comparison of the electronic structure of Amavadin and related complexes on the one hand, to VO^{2+} complexes on the other, suggests an electronic equivalence between the bonding of two mutually *trans* η^2-*N,O* groups and one oxo-group.[5] Thus, the dominant bonding interaction in each case is axial and leads to the d-obital sequence $d_{xy} < d_{xz}, d_{yz} \approx d_{x^2-y^2} < d_{z^2}$. Thus, Amavadin displays d-d transitions and EPR properties similar to those of a VO^{2+} centre.

1. H. Kneifel, E. Bayer, *Angew. Chem. Int. Ed. Engl.*, 1973, **12**, 508.
2. H. Kneifel, E. Bayer, *J. Am. Chem. Soc.*, 1986, **108**, 3075-3077.
3. M.A. Nawi, T.L. Reichel, *Inorg. Chim. Acta*, 1987, **136**, 33-39.
4. E.M. Armstrong, R.L. Beddoes, L.J. Calviou, J.M. Charnock, D. Collison, N. Ertok, J.H. Naismith, C.D. Garner, *J. Am. Chem. Soc.*, 1993, **115**, 807-808.
5. D. Collison, R.J. Deeth, S.N. Ertok, C.D. Garner, submitted for publication.

HEME CATALYTIC PROPERTIES IN PLANT AND FUNGAL PEROXIDASES

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The catalytic properties of heme which is an active center of heme-containing plant and fungal peroxidases is significantly affected by the structure of a polypeptide chain coordinating the heme moiety and forming the substrate-binding pocket. The concentration of peroxidase active centers can be easily estimated using pyridine-hemochrome complex formation. The turnover numbers using ABTS as a substrate for six native and three recombinant peroxidases obtained in the laboratory have been determined. The highest values have been shown for native anionic tobacco and cationic horseradish peroxidase (1300 sec^{-1}) and the recombinant horseradish peroxidase expressed in E.coli (2300 sec^{-1}). The difference in heme catalytic properties between the native and recombinant horseradish peroxidase is accounted for the heme inactivation in the native preparations by modification of radicals of the oxidized substrates in contrast to the undamaged heme moiety in the recombinant enzyme produced in vitro. Two single-point mutants of recombinant horseradish peroxidase (Phe41->His neighbouring distal histidine and Phe143->Glu at the entrance of the substrate-binding pocket) demonstrate a dramatic loss of the catalytic activity (16 and 65 sec^{-1} respectively).

EPR, ENDOR and ESEEM Study of the Ni-Site of Soluble NAD$^+$-Reducing Hydrogenase from *Alcaligenes eutrophus*

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Hydrogenases catalyze the reversible two-electron oxidation of molecular hydrogen, they are important in the anaerobic metabolism of several bacteria [1]. Like other [NiFe]-hydrogenases the soluble NAD$^+$-reducing hydrogenase from *Alcaligenes eutrophus* contains a Ni-site and several iron-sulphur centers [2].

After reduction with NADH, this enzyme shows a typical Nickel EPR spectrum ("Ni-C") with g-components 2.20, 2.13 and 2.01. Illumination of the sample at low temperatures ($T < 100$ K) leads to an altered EPR spectrum, termed "Ni-L" ($g = 2.27, 2.10, 2.05$). The Ni-C spectrum can be completely recovered by warming the sample in the dark ($T > 130$ K). A similar behaviour was observed in other Ni-Hydrogenases [3]. Hyperfine splittings due to ^{61}Ni ($I=3/2$) are observed in hydrogenases from bacteria grown on ^{61}Ni-enriched media.

In order to obtain additional information on the structure of the Ni site and its changes upon illumination, ENDOR experiments were performed on both, the Ni-C and the Ni-L state of the enzyme. ^{1}H-ENDOR spectra were recorded for different B_0 field values covering the whole range of the EPR spectrum. At each field value only a distinct set of molecular orientations contributes to the ENDOR spectrum. This "orientational selection" in ENDOR of frozen solutions allows, in principle, to determine the anisotropic hyperfine coupling tensors by comparing the experimental data with powder ENDOR simulations [4]. Using the approximation of a point-dipolar interaction between the unpaired electron and the protons, the positions of protons relative to the g-tensor axes can be estimated.

The most obvious change in the ENDOR spectra between Ni-C and Ni-L is the disappearence of the signals from the largest proton hyperfine coupling (A=16-18 MHz) upon illumination. Signals from smaller hyperfine couplings (A=6-8 MHz) are detected in both species. A number of weakly coupled protons ($A < 5$ MHz) also contribute to the spectra, their signals could not yet be assigned to individual hyperfine couplings.

Similar experiments were performed on hydrogenase preparations in deuterated solvent in order to probe whether the detected protons are exchangeable.

In electron spin echo envelope modulation (ESEEM) experiments ^{14}N hyperfine couplings have been observed. However, the interpretation of ^{14}N–ESEEM data is difficult due to the quadrupole moment of the ^{14}N nuclei. To obtain more information on nitrogen hyperfine couplings experiments on ^{15}N labeled hydrogenases are in progress.

References

[1] ADAMS, M. W. W., *Biochim. Biophys. Acta* **1020** (1990) 115–145; PRZYBYLA, A. E., ROBBINS, J., MENON, N., and PECK JR., H. D., *FEMS Microbiol. Rev.* **88** (1992) 109–136.

[2] SCHNEIDER, K., CAMMACK, R., SCHLEGEL, H. G., and HALL, D. O., *Biochim. Biophys. Acta* **578** (1979) 445–461.

[3] VAN DER ZWAAN, J. W., ALBRACHT, S. P. J., FONTIJN, R. D., and SLATER, E. C., *FEBS Lett.* **179** (1985) 271–277.

[4] GREINER, S. P. and KREILICK, R. W., *J. Magn. Reson.* **100** (1992) 43–59.

The Chemistry and Antitumor Properties of Pt Anthraquinone Complexes

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Cis-diamminedichloroplatinum(II) (Cisplatin) is a well established antitumor agent which is in wide clinical use and is often administered in chemotherapy in conjunction with intercalative drugs such as adriamycin. Anthraquinones are the intercalating moieties of adriamycin and mitoxantrone and as such we chose to study Pt-anthraquinone complexes to determine their potential as antitumor agents. Towards that end we prepared several series of Pt-anthraquinnnone complexes and studied their structure-activity relationship. The complexes can be divided into two groups: first generation Pt complexes and second generation Pt complexes. (see figure). In the former the Pt has a N_2Cl_2 coordination sphere and is irreversibly tethered to anthraquinone while the latter has the carboplatin coordination sphere and the intercalators serve as a carrier molecule. The antitumor properties of the complexes are very sensitive to the structure. The antitumor activity depends on the ratio of Pt to intercalator; on the position of substitution on the aromatic ring; on other substituents on the ring; on the length of the linker chain and the on size of the Pt chelating ring. We studied the correlation between the antitumor activity and the *in vitro* DNA binding properties of the complexes and we also studied the correlation between the activity of the anthraquinone moiety and the activity of its Pt complex.

First generation analogs

Second generation analogs
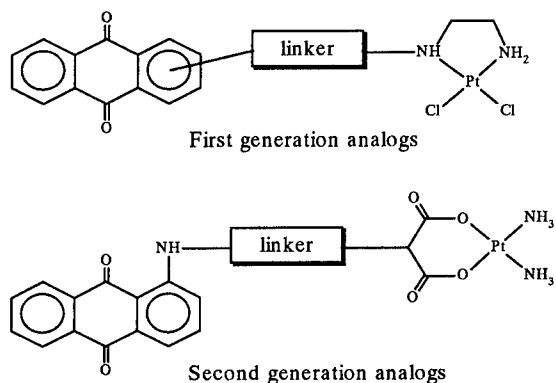
Theoretical study of a model of
manganese superoxide dismutase active site.

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The model of manganese superoxide dismutase active site considered in this Ab Initio theoretical study includes the metal cation and four ligands, namely three imidazoles and a formic acid/formate anion. The calculations have been carried out for the two oxydation states of the metal cation (Mn II and Mn III) and for the different possible spin states of the system.

The results obtained show that for both degrees of oxydation the system adopts the hight spin state. This result imply the occurence of two intersystem crossings during the catalytic cycle. This feature might, to some extend, be responsible for the lower activity of Mn SOD and its biomimetic models[1] when compared to Fe and Cu/Zn SOD for which no spin crossover is required.

The calculations also suggest that the ionization state of the coordinated aspartate carboxylic group changes during the catalytic cycle.

1: a) Pick, M.; Rabani, J.; Yost, F.; Fridovich, I.; *J. Am. Chem. Soc.* **1974**, *96*, 7329. b) Mc Adam, M.A.; Fox, R.A.; Lavelle, F.; Filden, E.M. *Biochem. J.* **1977**, *165*, 71. c) Kitajima, N.; Ozawa, M.; Tamura, N.; Moro-oka, Y.; Hirano, T.; Hirobe, M.; Nagano, T. *Inorg. Chem.* **1993**, *32*, 1879. d) Riley, D.P.; Weiss, R.H. *J. Am. Chem. Soc.* **1994**, *116*, 387.

FINE-STRUCTURE FLUORESCENCE SPECTRA OF MODEL COMPOUNDS OF CHLOROPHYLL AND THEIR INTERPRETATION

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The method of obtaining fine-structure fluorescence spectra of molecules in isotropic media based on the usage of selective laser exitation and helium temperatures is most advantageously applied in studying chlorophyll and its analogs.

The fine-structure fluorescence spectra and IR spectra of model compounds of chlorophyll - Zn octaethylporphyrin, Zn octaethylchlorin and derivative of Zn octaethylporphyrin with the isocyclic substitution are obtained. Its interpretation on the basis of the normal coordinate calculations is given.

Vibrations of substituents are observed in the spectra of substituted chlorins. Thus, a vibronic spectrum of Zn octaethylchlorin is predominantly formed by the vibrations of ethyl groups. Their activity is due to the mixing of macrocycle modes and modes of peripherial substituents with close frequencies. It should be noted that unfluence of central metal atom on the vibrations of hydrogenated ring differ from influence on the other ones. In particular frequencies 1194 and 1232 cm^{-1} decrease with substitution of Zn atom for stronger comlexing Ni atom, though, on other hand, the similar frequencies of metallooctaethylporphyrins increase.

According to the calculation results isocyclic substitution leads to symmetry disappearance of the majority of the vibrations. For instance, CC bond of methine bridge neighbouring with cyclopentane ring gives 50% contribution to the potential energy distribution. Vibrations of cyclopentane ring have frequencies in 830-890, 1000-1100, 1190-1210 cm^{-1} regions. These modes belong to the part of the cyclopentane ring, which does not interact with π-electronic system of porphyrin molecule, and therefore are not active in vibronic spectrum. CC stretching modes of this ring are delocalized and mixed with another modes of porhyrin macrocycle and there arise vibronic lines 644, 694, 915, 1178, 1239, 1424 and 1539 cm^{-1}.

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Bacterial nitric oxide reductase, a novel cytochrome *bc* complex

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The primary structure of nitric oxide reductase (NOR) from *Pseudomonas stutzeri* has been deduced from the *norCB* genes. The enzyme complex consists of 16.5 and 53 kDa subunits, associated with heme *c* and heme *b*, respectively. The mature cytochrome *c* subunit is a bitopic protein with a single membrane anchor and the bulk of the protein being periplasmic. The cytochrome *b* subunit is a strongly hydrophobic protein, arranged regularly as 12 transmembrane helices. The topology of NOR is supported by phosphatase gene fusions in *norC* and *norB*. Purified NOR contains cardiolipin, phosphatidylglycerol and phosphatidylethanolamin. Phospholipid is required for high catalytic activity with cardiolipin or phosphatidylglycerol increasing NOR activity by a factor of up to 5. Free fatty acids inhibited NOR, oleic acid showed the most pronounced effect. Detergents substituted for the phospholipid requirement of NOR. The highest specific activity of 60 μmol NO min^{-1} mg^{-1} was obtained after electrophoresis in the presence of laurylpropanediol- 3-phosphorylcholine. The enzyme as isolated exhibited a complex set of EPR resonances at low magnetic field with a prominent peak at $g = 6.34$ resulting from Fe(III) high-spin cytochrome *b*. The second prominent feature arose from a low-spin Fe(III) heme center with strong lines at apparent g values of 3.02 and 2.29, and a broad resonance at $g \approx 1.5$ which we assigned to the cytochrome *c* component of the enzyme. A ratio close to 1:1 for the low/high-spin heme centers in NOR was estimated. Reduced NOR incubated with NO gave the characteristic Fe(II) nitrosyl triplett centered at $g \approx 2.01$, with a hyperfine splitting of 1.70 mT. Alignment of the primary structure of NorB with that of bacterial *aa*$_3$ and *cbb*$_3$ oxidases, including FixN of *Bradyrhizobium japonicum*, revealed homology of NorB to the oxidase protein family (van der Oost et al., 1994).

Zumft, W. G., Braun, C., and Cuypers, H. 1994. Eur. J. Biochem. 219: 418-490.
Kastrau, D. H. W., Heiss, B., Kroneck, P. M. H., and Zumft, W. G. 1994. Eur. J. Biochem., in press.
van der Oost et al. 1994. FEMS Microbiol. Lett., in press.

Reductive dehalogenation of alkanes and alkenes by cobalamin

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Halogenated compounds are among the most important products of the chemical industry. They are used as solvents, intermediates, preservatives and pesticides. Because of their widespread use they can enter different environments and become ubiquitous pollutants. In anoxic environments, a broad variety of bacteria (e.g. methanogens, sulfate reducers, acetogens) have been shown to reduce aliphatic halogenated compounds cometabolically. Enzymes containing tetra-pyrrole cofactors are probably involved in these dehalogenation reactions. In several studies, it has been shown that reductive dehalogenation of different chlorinated methanes, ethenes and ethanes can be catalyzed by free tetra-pyrrole cofactors such as hematin, cobalamin and factor F_{430}.

The exact mechanism of these reductive dehalogenations is yet unknown. In our study kinetic and spectroscopic investigations will be carried out to elucidate if a one-step two-electron transfer or a two-step one-electron transfer is involved in the reductive dehalogenation by cob(I)alamin. The mechanisms of the reductive dehalogenations catalyzed by free or enzyme-bound cobalamin shall also be compared. The reactions are carried out in a buffered medium containing a reducing agent such as titanium (III) citrate or dithiothreitol. First data of the reductive dehalogenation of alkanes and alkenes catalyzed by free cobalamin will be presented and discussed in terms of a mechanistic model.

MONO- AND DI-NUCLEAR COMPLEXES OF MANGANESE(II) WITH 2,6-DIMETHOXYBENZOIC ACID AND NITROGENOUS BASES

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The role of manganese in biological systems is related to the ability of the element to yield polynuclear complexes where various oxidation levels, involving single-atom oxidation states in the range II-IV, are accessible. Oxide or hydroxide groups act as bridging ligands in such complexes, whereas the terminally ligated donors are O- or N-atoms from amino acid side chains.

Among dinuclear structures containing two Mn(II) ions, which have been proposed as reliable models of pseudocatalase and ribonucleotide reductase (RR) as well as useful precursors to higher nuclearity clusters, particular attention has been paid to water- and/or carboxylate-bridged complexes where metal-metal distances spanning from 3.3 to 4.3 Å have been observed, compared to the value of 3.6 Å in the catalase and manganese-reconstituted RR.

In the course of a study on carboxylate complexes of biological and environmental relevance, we isolated a ternary complex, formed by manganese(II), 2,6-dimethoxybenzoic acid and 2,2'-bipyridine. The striking structural feature of this complex is a dinuclear arrangement, arising from a rather unusual one-oxygen bridging mode of the carboxylate ligand. The molecular structure, as determined by single crystal X-ray diffraction, and the spectral properties of this dimanganese(II) adduct and those of an analogous monomeric complex, are presented here.

Zinc binding to peptides monitored by potentiometric titrations

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Zinc is a structural component in many proteins playing an important role for the folding and the tertiary structure of the polypeptide chain. We are trying to gain information on the factors governing the coordination behaviour of zinc by studying zinc complexes of small peptides. Peptide synthesis and structure determination by X-ray, EXAFS, and NMR methods are employed. This presentation deals with informations obtainable from potentiometric stability measurements.

According to the fact that zinc prefers binding to biological nitrogen and sulfur donors, histidine and cysteine containing peptides were the main object of investigation. Other amino acids in the peptides were chosen with respect to their coordinating ability (Asp, Lys) or to their occurrence in protein β loops (Gly, Pro, Val).

In this work the stability constants and hence the species distributions in aqueous solutions of zinc complexes of cyclic peptides (cylclo-GlyCys and cyclo-HisHis), open chain dipeptides with C-terminal Cys (AcGlyCys, AcLysCys, ZAspCys, ZAspHis, ZAspHisOMe, GlyCys-OEt, ValHisOEt), with N-terminal Cys (AcCysVal), open chain tripeptides (GlyGlyCysOEt, AcCysValCysOEt, AcHisValCysOEt, AcHisValHisOEt, BzCysGlyHisNH$_2$, BzHisGlyHisNH$_2$), and one open chain tetrapeptide (BzHisLeuGlyHisNH$_2$) have been determined and compared. The figures below show the species distribution of the zinc complexes of a selected peptide (L = GlyGlyCysOEt) and the proposed structure of the neutral complex ZnL$_2$.
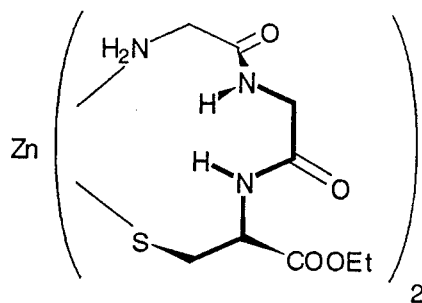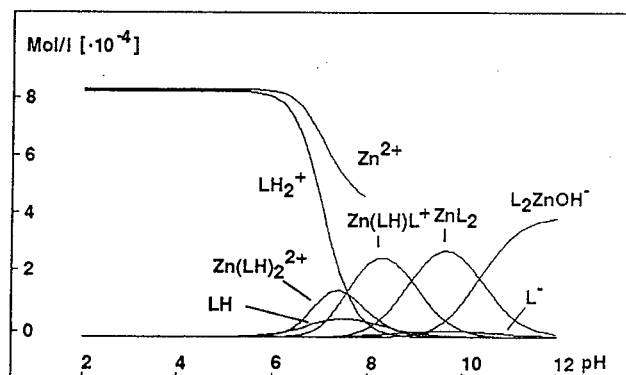
From the results obtainded an understanding of the mutual influences between the zinc ions and the peptide constituents is emerging.

Modelling Iron Biomineralisaton Processes

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Iron(III) oxyhydroxide and oxide minerals are the product of the hydrolysis of iron(III) salts. In biological systems this mineralisation process can be controlled by organic templates such as amino acid side-chains to produce biominerals capable of fulfilling specific functions. This process can be studied *in vitro*. In the presence of chelating ligands the hydrolysis can be suppressed and intermediate polyiron oxyhydroxide complexes can be isolated. The extent of the aggregation of the iron(III) centres is dependent on the amount of chelating ligand available and the hydrolysis conditions. These factors have been explored using a number of related ligands and hydrolysing bases.

In this way it is possible to isolate polyiron oxyhydroxide complexes with the general formula $\{Fe_xL_n(O)_y(OH)_2(H_2O)_p\}$. The number of iron atoms in the clusters range from three to nineteen and such examples of these clusters include hexanuclear iron-oxo cores such as $[Fe_6(\mu_3\text{-}O)_2(\mu_2\text{-}OH)_6(ida)_6]^{4-}$ and clusters containing 17 and 19 iron atoms which prove to be good models for the ferritin core.

A number of different ligands such as nta $(N(CH_2COOH)_3)$, ida $(HN(CH_2COOH)_2)$ and heidi $(H_3heidi= N(CH_2COOH)_2(CH_2CH_2OH))$ have been used to trap out iron oxyhydroxide complexes and spectral analyses have been performed to study the formation of the iron-oxo bridges within the complexes.

Clusters of different solubilities can be synthesised, for example the first reported examples of iron/heidi clusters were rather insoluble, but recent syntheses have resulted in the isolation of extremely soluble materials. These are currently being characterised by magnetic and spectroscopic measurements in order to ascertain whether they are larger iron oxyhydroxide aggregates and therefore better models for biominerals such as ferritin.

Reactions of FeMoco with chalcogeno-phenols and dithiols.

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FeMoco, the generally accepted site where N_2 is bound and reduced in nitrogenase [1], can be extracted from the enzyme from *Klebsiella pneumonia* (Kp) or other prokaryotes into N-methylformamide (NMF) and stored under strictly anaerobic conditions. In accordance with recently published results of X-ray single crystal analysis [2] we propose that in this process NMF substitutes the positions of the amino acids $Cys^{\alpha275}$ and $His^{\alpha442}$ of a tetrahedrally co-ordinated Fe and the octahedrally co-ordinated Mo atom of protein-bound FeMoco respectively.

We have studied the reactions of isolated FeMoco with thio- and selenophenol as well as with 1,2-benzene-dithiol and 3,4-toluene-dithiol. The structural changes of FeMoco were monitored by EPR, EXAFS, NMR and UV/vis spectroscopy.

Both chalcogeno-phenols react with FeMoco as demonstrated by the narrowing of the $S=^3/_2$ lines in the EPR spectra. The structurally intact FeMoco retains its activity. Titration experiments indicate a 1:1 stoichiometry for the binding of the phenols. The binding site for these chalcogeno-phenols is not the single Mo atom in the FeMoco cluster but a single Fe atom, most likely that bound to $Cys^{\alpha275}$ in the protein. Fe- and Se-K edge EXAFS show an Fe-Se distance of 2.36 ± 0.02 Å and Fe-C(aromatic) distances of 3.01 ± 0.02 Å (thiophenol) and 3.25 ± 0.02 Å (selenophenol). Parallel Se-K edge EXAFS measurements on a substituted selenophenol and FeMoco isolated from *Azotobacter vinelandii* are consistent with our work[3].

The greenish FeMoco reacts with the *ortho*-dithiols to form deep red solutions ($\epsilon_{489} \sim 10^4$ lmol^{-1}cm^{-1}) with precipitation of S_8. Similarities in the EPR spectra of these products with that of FeMoco indicate that the products of these reactions are Fe-S clusters related to the FeMoco cluster.

[1] T. R. Hawkes, P. A. McLean, B. E. Smith, *Biochem. J.* (1984) **217**, 317. [2] M. K. Chan, J. Kim, D. C. Rees, *Science* (1993) **260**, 792. [3] S. D. Conradson, B. K. Burgess, W. E. Newton, A. Di Cicco, Z. Y. Wu, C. R. Natoli, B. Hedman, K. O. Hodgson, *Proc. Natl. Acad. Sci. USA* (1994) **91**, 1290.

Donor-acceptor charge equilibria in Photosystem II.

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The terminal electron acceptor of Photosystem II (PSII) is a linear $(Quinone)_A Fe^{2+}(Quinone)_B$ complex. If Q_B is replaced with inhibitory molecules (e.g. DCMU), the state $Q_A^- Fe^{2+}$ recombines slowly with an appropriate oxidised donor. There are two heterogeneous populations of the state $Q_A^- Fe^{2+}$ characterised by EPR signals at g=1.9 and g=1.82, and a number of likely recombination partners on the donor side. A recent EPR and thermoluminescence (TL) study [1], indicated that in untreated PSII particles, excited by light 200K, the S_2 state of the water-splitting Mn-enzyme recombines with the g=1.9 $Q_A^- Fe^{2+}$ form resulting in the appearance of the Q (TL) band. The g=1.82 $Q_A^- Fe^{2+}$ signal, on the other hand, recombines slowly with Signal II_{slow} (tyrosine Y_D^+). We have extended these studies and observed the following:

In manganese-depleted PSII particles illuminated at 200K, or in intact PSII particles illuminated at 77K, the light-induced g=1.9 signal decays at +25°C, in parallel with Signal II_{slow}. Furthermore, DCMU addition in manganese-depleted preparations results in the appearance, in the dark, of a small g=1.9 signal, due to back electron donation from Q_B^- to Q_A. This signal also decays in parallel with Signal II_{slow}. Absence of DCMU, or oxidation of Q_B^- prior to DCMU addition results in the absence of a g=1.9 and a stable Signal II_{slow}.

The fast phase of the biphasic g=1.82 decay [1] does not occur in Tris-treated, or intact PSII particles illuminated at 77K. It appears after illumination of the latter at ≥200K. Involvement of the water-splitting enzyme in the recombination pattern of the fast component of the g=1.82 is therefore plausible.

[1] Demeter et al. (1993), FEBS Lett. 336, 352-356

Red-ox reactions of the iron site of ribonucleotide reductase

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Ribonucleotide reductase catalyses the reduction of ribonucleotides to the corresponding deoxyribonucleotides, necessary for DNA synthesis. The small subunit of iron-containing ribonucleotide reductases, protein R2, contains an oxo-bridged antiferromagnetically coupled diferric center and a stable free radical on a tyrosine residue (Y122) in its active state. EPR spectroscopy has been used to characterize the fully reduced and the mixed valent states of the iron site in mouse and herpes simplex virus protein R2 (1). The mixed valent state can be stabilized at room temperature in these proteins, in contrast to E. coli protein R2. The interaction between the diferric site and the tyrosyl radical has been studied in active protein R2 from mouse and herpes simplex virus using saturation recovery EPR (2).

The iron/radical site is formed in apoprotein R2 by a red-ox reaction with ferrous iron and oxygen. The corresponding reaction has been studied in an E. coli mutant apoprotein R2, Y122F, which lacks the essential Y122. The normal iron center is formed, but the reduction equivalent from Y122 now must be supplied elsewhere. EPR spectroscopy at room temperature and low temparature shows several paramagnetic species appearing on a sec-min time scale (3). Performing the ferrous iron/oxygen reaction with Y122F apoprotein R2 containing deuterium labeled tryptophan or tyrosine residues shows that at least two species can be assigned to tryptophan free radicals. Apparently the red-ox reaction leading to formation of the metal site creates a strong oxidation potential in the interior of the protein, leading to oxidation of tryptophan side chains. In the absence of external reductants they exist on a time scale of minutes. Their sites may represent potential participants in electron transport pathways needed for the enzymatic function.

1. Atta, M., Andersson, K.K., Ingemarson, R., Thelander, L. and Gräslund, A. (1994), submitted.

2. Galli, C., Andersson, K.K., Atta, M., Thelander, L., Gräslund, A., and Brudvig, G.W. (1994), in preparation.

3. Sahlin, M., Lassmann, G., Pötsch, S., Slaby, A., Sjöberg, B.-M. and Gräslund, A. (1994) J. Biol. Chem., in press.

The Application of Protein Engineering to the Study of the Mechanism of *Pseudomonas aeruginosa* Cytochrome c Peroxidase

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Cytochrome c peroxidase (CCP), a 36kD monomer, from *Ps.aeruginosa* catalyses the oxidation of Cu(I) azurin by hydrogen peroxide. It contains two c-type haems with widely separated redox potentials (+320mV and -330mV). One function of azurin is to **activate** the resting oxidised enzyme by reducing the high potential haem to produce a half-reduced species. This is an intermediate in the **catalytic** cycle (Figure 1) in which azurin also serves as an electron donor[1]. In order to study the determinants of protein/protein recognition and the mechanisms of electron transfer we propose to construct a collection of variant azurins which we will analyse by rapid reaction kinetics and stopped-flow spectroscopy.

Azurin has successfully been overexpressed and purified in large quantities. Two variant genes have been constructed by PCR mutagenesis. These will be cloned into the vector pKK233,3 and expressed to produce azurins with the amino acid changes M44K and F114I, both found in a hydrophobic patch on the protein surface which is believed to play an important role in electron transfer.

Figure 1

We have recently determined the entire DNA sequence of the CCP gene, and the derived amino acid sequence shows some differences to that previously published[2]. This will aid interpretation of existing X-ray crystallography data[3], which will be of great importance in designing a similar programme of mutagenesis and biophysical studies for CCP itself.
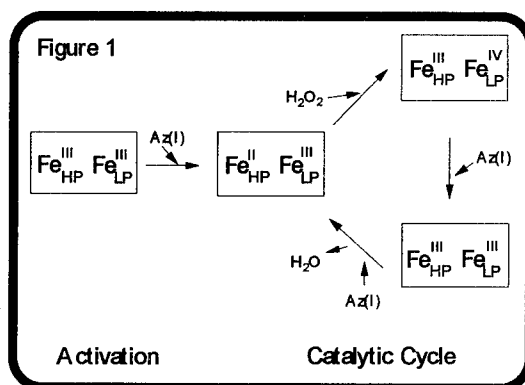
References
1. N.Foote, R.Turner, T.Brittain, C.Greenwood, *Biochem.J.*, **283**, 839 (1992)
2. M.Ronnberg, N.Kalkinnen, N.Ellfolk, *FEBS*, **250**, 175 (1982)
3. V.Fulop, R.Little, A.Thompson, C.Greenwood, J.Hajdu, *J.Mol.Biol.*, **232**, 1208 (1993)

Directed Multi-Heme Self-Assembly and Electron Transfer
in Model Phospholipid Membranes

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Abstract. Vectorial processes in biological systems such as the energy transduction strategies of respiration and photosynthesis occur in intricately constructed membrane assemblies.[1,2] We have sought to explore the deployment of artificial hemes in phospholipid vesicles to extend the limits of membrane-directed self-assembly and to probe the nature of electron transfer within these assemblies. In this lecture strategies for the construction of model membrane systems will be described. A synthetic membrane spanning bis-heme assembly **3** has been constructed from the steroidal porphyrin **1** and an amphiphilic zinc porphyrin **2**. Anionic sites on **2** serve to form a receptor site which recruits cytochrome *c* to the outside surface of phospholipid vesicles to form a robust adduct **4** with a high binding constant. The structure of the construct has been probed with fluorescence techniques and by observing electron transfer between cytochrome *c* and the redox center embedded within the phospholipid bilayer at an approximately known distance. The observed electron transfer rate at a known driving force is suggestive that distances and structural information may be deduced from the surprisingly simple behavior of these complicated assemblies.
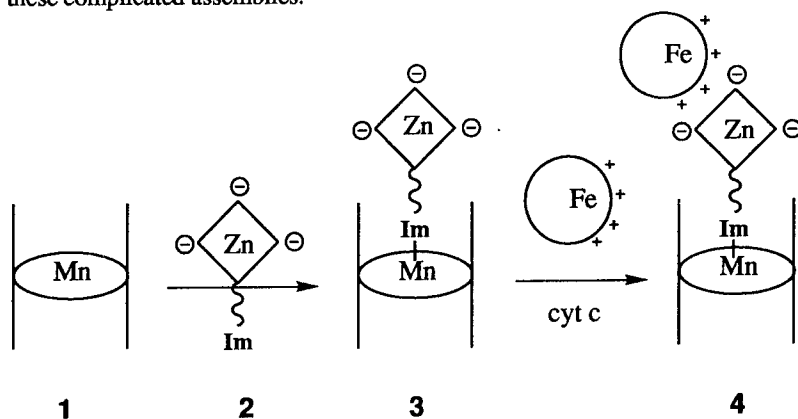
1. Ungashe, S. B. and Groves, J. T. "Porphyrins and Metalloporphyrins in Synthetic Bilayer Membranes", in *Advances in Inorganic Biochemistry*, Vol. 9, L. G. Marzilli, Ed., Academic Press, **1993**, 318-351.
2. Groves, J. T., Fate, G. D., Lahiri, J. *J. Am. Chem. Soc.*, **1994**, *116, in press*. (June).

Synthesis, spectroscopic characterization and "in vitro" cytotoxic activity of some novel Pt(II) complexes

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In this work we present a series of complexes, analogous to $cis(NH_3)_2PtCl_2$, containing, instead of the classic amino group, a bulkier aromatic residue, capable of linking DNA through non covalent interactions.
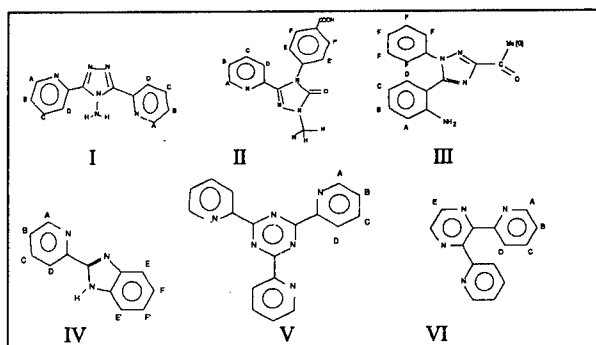
The ligands used throughout this work are listed on the left:

The following complexes have been synthesised:

1a) [4-amino-3,5-di-2-pyridyl-4*H*- 1, 2, 4-triazole (N,N)] (cis-$(NH_3)_2$Pt)]$^{++}$ $2NO_3^-$
1b) [4-amino-3,5-di-2-pyridyl-4*H*-1, 2, 4 - triazole (N,N)] $PtCl_2$
2) [2-metil-3-oxo-4-(o-carboxy-phenyl) 5-pyridyl-1,2,4 triazole (N,N)] $PtCl_2$
3) [1-phenyl-5(2-aminophenyl)-3-acetyl 1,2,4-triazole (N,N)] $PtCl_2$
4) [2-(2-pyridyl)benzimidazole(N,N)] $PtCl_2$
5) [2,4,6-tri(2-pyridyl)-1,3,5-triazine (N,N) (PtCl)]$_2^{++}$ $PtCl_4^=$
6) [2,3-bis(2-pyridyl)pyrazine(N,N,N,N)]$(PtCl_2)_2$

The novel compounds were characterised by molar conductivity, infrared and ¹HNMR spectral studies. Resonances assignments in compound 1a were confirmed with the aid of a ¹H¹H-COSY spectrum. In table 1 ¹HNMR data are listed.

Table 1	A	B	C	D	E,E'	F,F'	G	NH₂	Solv.
Ligand I	8.76q	7.54q	8.03dt	8.25q					CD₃OD
Complex 1a	8.68d 8.79d	7.84q 7.69q	8.45t 8.10t	8.88d 8.20d					D₂O
Complex 1b	8.61d 8.80d	7.84m 7.70m	8.42m 8.14m	9.42d 8.14m					DMSO
Ligand II	8.32d	7.40m	7.96dt	7.86d	7.95d	7.36d	3.52s		DMSO
Complex 2	9.58d	7.85dt	8.24dt	7.03d	8.32d	8.02d	3.90s		DMF
Ligand III	6.79d	6.46dt	7.16dt	6.79d		7.42m	4.04s	5.18s	CDCl₃
Complex 3	*	7.21t	*	7.11d		7.65m	4.01s	8.22s	DMF
Ligand IV	8.75d	7.75dd	8.02t	8.38d	7,54 m	7,26m			MeOH
Complex 4	9.44d	8.34dd	8.44t	8.77d	7,80m	7,51t 7,42t			DMSO

Table 2	ID₅₀
Cisplatinum	1.2
Carboplatinum	5.4
Complex 1b	10.9
Complex 3	11.5
Complex 5	3.5
Complex 6	3.6

The complexes were tested for "in vitro" cytotoxic activity against human tumor cell line HL60. In Tab. 2, ID_{50} values for the most active complexes (1b, 3, 5, 6) are summarised. The new complexes, especially 3 and 5, are a typical example of Platinum(II) compounds which are active, although they don' t contain primary or secondary amine linked to the metal center. Hence they could posses a different mode of action toward DNA, possibly by intercalating the aromatic ligand between base pairs.

Intercations of Water Soluble Porphyrins with Biological Polymers. Aggregation and Molecular Recognition.

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Evidence that cationic porphyrins can interact with DNA was first presented by Fiel and co-workers[1,2]. Since these original reports, interactions of porphyrins with DNA have been characterized utilizing a wide range of techniques and a detailed picture of the modes of the binding has emerged.[3] In addition to territorial binding (virtually observed for any positively charged species), cationic water soluble porphyrins as tetrakis-(4-N-methylpyridyl)porphine (H_2TMpyP-4) and some of its metallo-derivatives have been shown to intercalate and/or bind externally to DNA. Porphyrin assemblies on duplex DNA have been also reported, but thus far, only for those derivatives such as trans-bis(N-methylpiridinium-4-yl)diphenylporphine (t-H_2Pagg) which have tendency to self-aggregate in aqueous solution.

Recently, we have shown that H_2TMpyP-4 and its Copper(II) and Zinc(II) derivatives interact with single-stranded synthetic polyribonucleotides, and that these porphyrins can be "trapped" during the formation of a double helix, forming inclusion-like transient complex. In addition, in the presence of poly(A) both H_2TMpyP-4 and CuTMpyP-4 display induced circular dichroism spectra in the Soret region, whose shape and intensity indicate the aggregation of the porphyrins on the polyribonucleotide chiral matrix, *although neither porphyrins tends to aggregate when free in solution.* Interestingly, the spectroscopic features of the analogous complex of H_2TMpyP-4 with poly(dA) indicates a non-aggregated, externally bound porphyrin. Therefore our results are consistent with the following conclusions: i) in spite of their low tendency to aggregate H_2TMpyP-4 and CuTMpyP-4 form assemblies on single stranded poly(A); ii) the formation of these assemblies is *specific* in that these porphyrins discriminate the A-form of polynucleotides [poly(A)] from the B.form [poly(dA)].

Aggregation of H_2TMpyP-4 and CuTMpyP-4 on α-helical polypeptides will be also discussed.

References.

1. R. Fiel, J. C. Howard, E. Mark, N. Datta-Gupta, Nucleic Acid Res., **1979**, *6*, 3093.

2. R. Fiel, B. R. Munson, Nucleic Acid Res., **1980**, *8*, 2385.

3. R. F. Pasternack, E.J. Gibbs, ACS Symp. Ser., **1989**, *402*, 59

UNIFYING CONCEPTS IN IRON-SULFUR BIOCHEMISTRY

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Thirty-five years of research on iron-sulfur proteins has produced an encyclopedic body of data. Fe/S clusters are present in many different, perhaps all, living cells. They exert a range of functions in many different processes. They exhibit a rich variety of structures and flexibilities. Recent results on larger clusters with complicated chemistry and magnetism have incited me to attempts at ordering this knowledge by means of a few simple, predictive concepts. In particular, I wish to consider an 'Aufbau' principle for Fe/S clusters that affords the ordering of different Fe/S structures in a seniority scheme such that an increased structural complexity goes parallel with an increase in redox flexibility, catalytic versatility, structural convertibility, demand on the biosynthesis machinery.

The typical tetrahedral coordination and weak-field to intermediate-field ligands make the Fe in Fe/S clusters always high-spin, and this holds true even with higher coordination number by external ligands. Thus, Fe/S clusters are modular units from four building blocks: $hs\text{-}Fe^{3+}$, $hs\text{-}Fe^{2+}$ (alternatively: e^-), $\mu\text{-}S^{2-}$, and external ligands. Therefore, the magnetism of Fe/S clusters is completely interpretable in terms of exchange interactions between spins that can be 5/2 or 2. This explains the success of magnetic spectroscopy in identifying biological Fe/S structures.

The previous also suggests that we can order Fe/S structures by generating increasingly complex structures from the starting structure [2Fe(III)-2S]4Cys by adding and/or substituting building blocks. In its turn this building-up process suggests the following hypotheses for Fe/S biochemistry: 1) increasing the number of Fe ions increases the number of possible redox states but also decreases the ΔE_m between subsequent steps, thereby increasing the chances for multi-electron processes; 2) a more complex structure means increased potential for tailor-making asymmetric Fe sites for catalysis; 3) a more complex structure means increased potential for regulation by cluster conversion; 4) increased cluster complexity is paid for by increased complexity of the biosynthetic factory.

New data on a variety of Fe/S proteins will be presented that bear on the testing of these hypotheses.

Is the Combination of Depronated Amides and Oximes as Ligand Donors well Suited for Stabilization of Trivalent Nickel ?

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Since the discovery of trivalent nickel in hydrogenase, the stabilization of this oxidation state has evoked considerable interest. It was demonstrated that the lowest redox potentials were achieved with anionic, highly polarizable ligand donors like thiolates, phenolates, amidates or oximates.[1] The largest stabilizing influence is exerted by oximates. In this study for the first time the combination of deprotonated amides with oximes is examined. As depicted by the general Scheme I, all the used ligands contain two amides and two oximes.
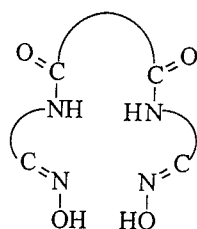
Scheme I
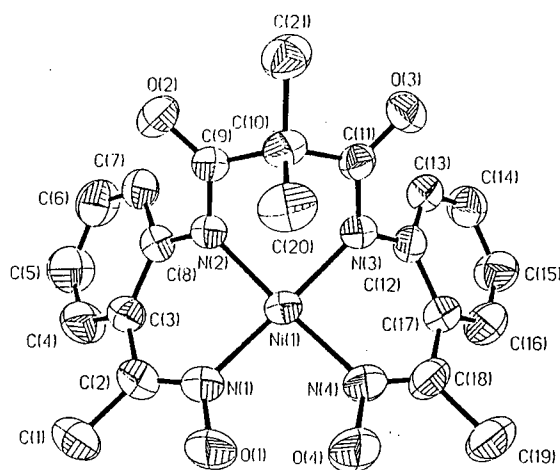
Figure 1

Nickel complexes were prepared with several representatives of this ligand type differing only in their chelate ring sizes with the coordinated metal. All nickel(II) complexes are diamagnetic and square planar. The crystal structure shown in Figure 1 is an example from all these complexes. A detailed electrochemical study on the redox potentials of this new type of nickel complexes is presented.

(1)   Krüger, H.-J.; Peng, G.; Holm, R.H. *Inorg. Chem.* **1991**, *30*, 734.

ESR Spectroscopy of Average-valence Docopper

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Recent EPR-detection of mixed-valence dicopper sites in metalloproteins[1] has prompted studies of model complexes. Formerly these have been restricted to the observation of 7-line spectra of solutions and glasses that become valence-trapped (4-line) at low temperature.

Three dicopper azacryptands have recently been structurally characterised[2]. The mixed-valence nature of the three complexes is evidenced by a 7-line hfs in the ESR spectra. The persistence of this spectrum to low (4K) temperature identifies the dicopper complexes as delocalised Class III compounds[3]. In all cases, the spectra show strong similarities: isotropic 7-line X-band spectra in fluid solution, anisotropy of dmf glass spectra in X- and Q-band, $g_\perp(\sim2.0) < g_\parallel(\sim2.15)$, $A_\perp(\sim10G)$, $A_\parallel(\sim100G)$ e.g. see Figure1. Structural similarities observed in these cryptates include local site symmetry for Cu (C_3) and a short Cu-Cu distance 2.41-2.45 Å, indicative of Cu-Cu bonding.

2500	3000	3500
	H / G	

11000	12000
	H/G
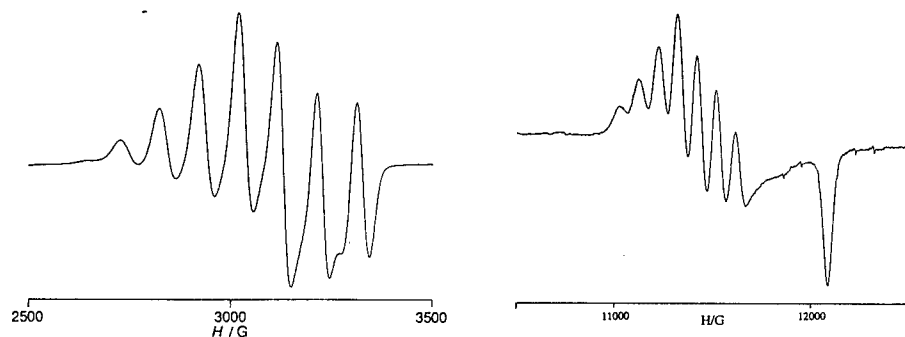
Figure 1   X-band DMF glass of **a**   Q-band DMF glass of **a**

Both X- and Q-band ESR spectra can be closely simulated. Analysis of the ESR spectra[4] gives an electron population in the magnetic orbital (d_{z^2} for trigonal bipyramidal copper) of about 0.35 e per Cu. Nitrogen hfs is not resolved in the spectra.

References.
1. (Cyt C Ox): B.G.Malstrom and R.Aasa, FEBS,(1993),**325**,49.
   (N₂OR):   P.H.M.Kroneck,W.A.Antholine,J.Reister and W.G.Zumft, FEBS Lett, (1988),**242**,70.; P.H.M.Kroneck,W.A.Antholine,H.Koteich,D.H.W.Kastrau,F.Neese and W.G.Zumft, 419, Bioinorganic Chemistry of Copper (Eds, K.D.Karlin and Z.Tyeklar),Chapman and Hall, 1993.
2. See adjacent poster. J.Nelson et al.
3.M.B.Robin and P.Day, Adv.Inorg.Chem.Radiochem.,(1967),**10**,247.
4. M.Symons, Chemical and Biochemical Aspects of Electron Spin Resonance Spectroscopy, Van Nostrand Rheinhold, 1978.

Ferritin : the importance of iron storage

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Iron is a relatively abundant element but, because of the insolubility of Fe(III), its bioavailability is poor. In spite of its biochemical versatility, it also has potential for harm, because it can promote the formation of highly toxic molecules. Hence, it is important for cells to have a means of iron storage and for cellular iron uptake, storage and utilisation to be tightly and co-ordinately regulated. In mammals, iron is delivered by transferrin and stored by ferritin. An iron regulatory factor (IRF) controls levels of the transferrin receptor and ferritin in response to iron, the former declining and the latter rising as iron levels increase. A likely intra-cellular iron transporter is citrate. The IRF converts to an active aconitase (by binding an Fe-S cluster), when iron is abundant, linking iron metabolism with the citric acid cycle.

Very little is known about how iron is stored in ferritin *in vivo*. Much of our work in Sheffield is directed to understanding iron storage mechanisms *in vitro* (in collaboration with other groups in Italy, Israel, France, Germany, Japan and America, as well as in Great Britain). Recombinant technology has greatly aided our research and Mössbauer spectroscopy has allowed detailed studies of the iron complexes formed. Mammalian ferritins contain two types of subunit, H and L. The subunits fold as 4-alpha-helix bundles and assemble as 24-mer shells with a cavity for iron storage as ferrihydrite. H subunits contain a di-iron site which catalyses Fe(II) oxidation giving μ-oxo-bridged Fe(III) dimers. Iron leaves the oxidation site and migrates into the cavity to form ferrihydrite clusters, which act as secondary sites for Fe(II) oxidation. Inter-molecular Fe(III) migration also occurs further enabling iron-core build-up. The di-iron centre of ferritin H chains resembles, to some extent, those of methane mono-oxygenase and ribonucleotide reductase. Near to this centre is a tyrosine, the role of which is under investigation. Both an Fe(III)-tyrosinate charge-transfer complex and tyrosine radicals are formed as iron is oxidised in ferritin.

The biosynthesis of ferritins in invertebrates, plants and bacteria is also stimulated by iron, but the regulatory mechanisms are different from those of mammals. Comparative studies of iron uptake, oxidation and storage within ferritin molecules from different species aids the understanding of these processes.

Polydentate Ligand Construction on Cobalt(III).

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The chemistry of amino acids bound to metal ions, particularly cobalt(III), has been the subject of much research for some considerable time. However, reactions which involve fragmentation of the amino acid ligands or redox chemistry of the metal ion can be difficult to study due to decomposition of the resulting complexes. We are attempting to build complexes suitable for such studies by incorporating amino acids into penta- and hexadentate ligands.

Our synthetic approach is to use the established procedure[1] of replacement of one of the chloro ligands of a polyamine cobalt(III) complex, eg. [Co(tren)Cl$_2$]$^+$ (tren = tris(2-aminoethyl)-amine), with another ligand which bears a carbonyl group suitable for an intramolecular condensation reaction. Condensation and reduction of the resulting imine gives a pentadentate ligand. A second ligand substitution-condensation-reduction cycle gives a hexadentate ligand. Different carbonyl containing ligands can be used at each step to produce a range of possible complexes. A 2-aminoethyl substituent can be added through use of aminoacetaldehyde (as an acetal), a π-acidic donor results from use of acetylpyridine, and amino acids can be incorporated through the use of the related ketoacid. It is found, however, that the order of addition of the fragments can be important, and that a variety of isomers can be produced.

+ isomers
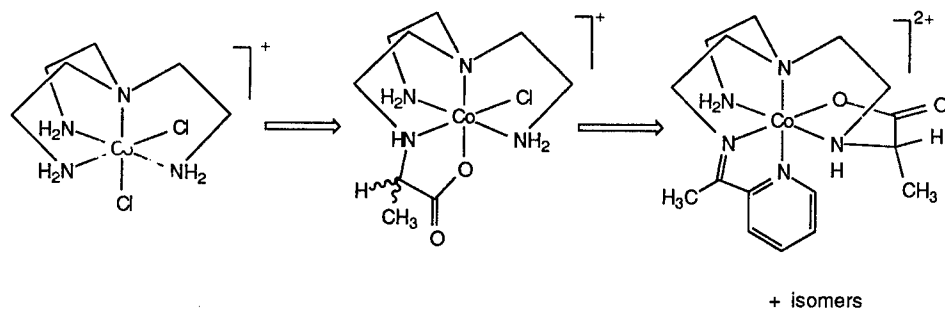
1. L.M. Engelhardt, A.R. Gainsford, G.J. Gainsford, B.T. Golding, J.MacB. Harrowfield, A.J. Herlt, A.M. Sargeson, A.H. White, *Inorg. Chem.*, 1988, 27, 4551.

Synthesis and Spectroscopic Characterisation of Selectively Deuterated Di-and Tetranuclear Manganese Complexes

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Di- and tetranuclear oxo-bridged manganese (III/III and III/IV) compounds can serve as model systems for the manganese unit in Photosystem II in cyanobacteria and higher plants.

EXAFS-data show, that the ligand-sphere of the four manganese atoms, consists mostly of O-donors, e.g. oxo- and carboxylate groups and probably only very few N-atoms. The ESR multiline spectrum of the S_2-state discovered by Dismukes and Siderer reveals that all manganese ions are electronically coupled and are in the oxidation-states + III and + IV.

We have synthesized novel bis (μ–oxo-)(μ-carboxylate) dimanganese compounds and measured their EPR and ENDOR (electron nuclear double resonance) spectra (Lubitz et al.). In order to obtain a better understanding of these spectra it was necessary to selectively deuterate these complexes. We here present the synthesis and characterisation of these species.

$[L(Mn^{III}(\mu\text{-oxo})_2(\mu\text{-acetato})Mn^{IV})](BPh_4)_2$
With L = (1,4,7 -triazacyclononane)-1,2-ethan
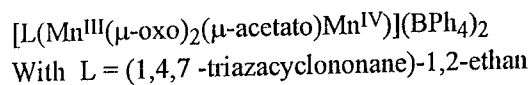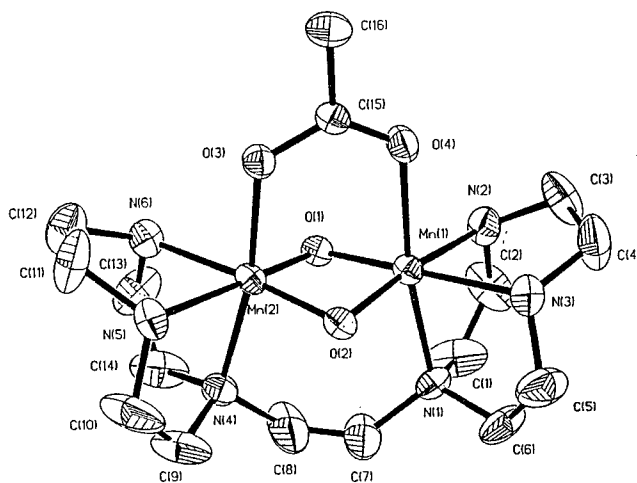
DIRECT ELECTROCHEMISTRY OF HIGH- POTENTIAL IRON-SULFUR PROTEINS

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The high-potential iron-sulfur proteins are a special class of ferredoxins, containing a [4Fe-4S] cluster bound to the protein by four cysteinyl sulfur ligands and buried in a hydrophobic environment. HiPIPs are usually but not exclusively found in purple photosynthetic bacteria [1]. The oxidized HiPIPs contain a one-electron reduced cubane $[4Fe-4S]^{3+}$ (i.e. $3Fe^{3+}Fe^{2+}$) cluster. The redox potential of the 3+/2+ transition ranges from +50 to +450 mV within this class of proteins. The overall charges range from very negative (−14) to positive (+4) [2].

The HiPIPs form a group of related and well-studied small redox proteins. This offers the opportunity to determine the influence of protein properties on redox potentials and interactions with the electrode using direct electrochemistry. Another interesting aim is to obtain the super-reduced $[4Fe-4S]^{1+}$ state normally present in reduced ferredoxins. We are investigating the HiPIPs from *Ectothiorhodospira vacuolata* (iso-1 and iso-2), *Chromatium vinosum*, *Rhodopseudomonas gelatinosa*, *Rhodospirillum tenue*, *Rhodophila globiformis* and *Rhodospirillum salinarum* (large isoenzyme).

As expected the charge of the protein has a large influence on the electrochemical response. We use a glassy carbon electrode with a negatively charged surface. At a pH below the pI of the HiPIP direct electrochemistry without a promotor is possible. Above the pI the response is poor and a positively charged bridging promotor like neomycin or poly-L-lysin is required. However, the response is not always stable. We found that the stability can be improved by adding morpholin in combination with negatively charged protein and poly-L-lysin or adding monomeric amino acids to the positively charged protein. These 'stabilizers' apparently prevent the blocking of the electrode by denatured protein during electrochemistry.

The slope of the midpoint potential *versus* the square root of the ionic strength varies between 0 and −40 mV/unit. The pH-dependence of the midpoint potentials of HiPIPs is not very strong. The maximum slopes observed are about −10 mV/pH. The coupling of electron and proton transfer is probably not direct (protonation near the cluster) but of an allosteric nature (far from the cluster). The dependence of the midpoint potential on the temperature varies strongly over the different HiPIPs. The different slopes and break points indicate that the conformational change upon reduction is not unique within the class of HiPIPs.

1   R.P. Ambler, T.E. Meyer and M.D. Kamen, Arch. Biochem. Biophys. 306 (1993) 215-222.
2   T.E. Meyer *et al.*, Proc. Natl. Acad. Sci USA 80 (1983) 6740-6744.

Catalytic site Cd-substituted horse liver alcohol dehydrogenase: Metal coordination geometry and protein conformation

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The coordination geometry of the catalytic site in Cd-substituted horse liver alcohol dehydrogenase has been investigated as a function of pH for the free enzyme (pH 7.9-11.5), and the E-NADH (pH 8.3-11.5) and E-NAD$^+$ (pH 5.8-11.4) complexes, using the method of perturbed angular correlation of γ-rays (PAC). A procedure for E-NAD$^+$ measurements, which are difficult due to small substrate contaminations combined with the high affinity of NADH for the Cd$_2$Cd$_2$LADH, is also presented. We find an apparent pK$_a$ of 11.0±0.3 for the free enzyme, probably due to ionisation of metalbound water. Angular overlap model (AOM) calculations of the nuclear quadrupole interaction paramaters (NQI), using the crystalstructure of the E-NADH-DMSO complex, agrees well with the measured NQI at low pH. At high pH agreement can only be obtained by a local conformational change involving the metal ligands Cys46 and Cys174, decreasing the S-Cd-S angle. According to AOM calculations, the coordination geometry of the metal in the E-NAD$^+$ complex is in the low S-Cd-S angle conformation at all pH values. The E-NADH complex produces no spectral changes within the pH-range, in good agreement with a pK$_a$ of 12 or more. The change in local conformation seems to be triggered by the presence of a negatively charged solvent metal ligand, suggesting that it is caused by electrostatic interaction.

X-ray analysis of metal binding to the ferritin of *Escherichia coli* (Ec-FTN) : first direct observation of the Fe^{3+} sites in a ferritin.

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Two ferritins have been isolated from *E.coli*; one (FTN) contains only non-haem iron, the other (BFR) also contains haem. FTN has been crystallised in a tetragonal space-group (I4, with a=b=13.0nm and c=17.3nm) and the structure has been determined. Soaking of the crystals with various metal salts has led to the identification of Fe^{3+}, Tb^{3+} and Zn^{2+} binding positions. X-ray analysis has located Fe^{3+} at a di-iron site (Fe-Fe distance 0.38nm) in the centre of the subunit. The molecular structure of FTN is similar to that of human H chain ferritin (HuHF) and its iron-binding centre closely resembles the postulated ferroxidase centre of HuHF. Tb^{3+} ions also bind at these centres in both ferritins. Mössbauer spectroscopic measurements show that FTN, like HuHF, catalyses the oxidation of Fe(II) with the formation, first, of Fe(III) μ-oxo-bridged dimers, and, later, of Fe(III) monomers and clusters. A monomeric Fe^{3+}-binding site, unique to FTN, which also binds Tb^{3+}, has been found on the cavity surface. In contrast, certain sites for Tb^{3+} and Zn^{2+} which are present in the threefold inter-subunit channels of HuHF (and which also bind monomeric Fe^{3+}) are absent from FTN. Mössbauer analysis of reconstituted FTN iron-cores has revealed a novel type of Fe(III) cluster along with ferrihydrite clusters. In similar experiments, HuHF gives only ferrihydrite. It is speculated that the novel cluster in FTN may form at the unique cavity metal site located by X-ray analysis. Further studies on the binding of Zn^{2+}, and the competition between Tb^{3+} and Zn^{2+} for Fe^{2+}/Fe^{3+} sites, are in progress.

Spin Relaxation N.M.R. Studies of Iron(III)-Ligand Systems

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The high paramagnetism of high spin d^5 iron(III) complexes makes it very difficult to obtain useful structural information using conventional ^1H and/or ^{13}C N.M.R. spectroscopies. This is because the nuclei of iron(III) ions behave like non-spherical, charged rotating bodies, and show line broadening in N.M.R. experiments. It is possible, however, to monitor the resonance of a solvent present in systems containing paramagnetic ions with short electronic relaxation times [1], and so gain information about the rate of solvent exchange occurring within such a system. The linewidth of the N.M.R. solvent signal is a source of information about the cation - solvent relationship. The linewidth can be determined from the spin-lattice, T_1, and/or spin-spin, T_2, relaxation times of a system.

Comparisons of the modified relaxation times in a metal - ligand system with those for pure solvent give an idea of the effect that dissolved ions have on the solvent. In aqueous systems, the T_1 time is of the order of one second, so the environment of a water molecule changes many times within the T_1 interval.

Oakes and Smith [2, 3] developed a method to determine solvation numbers for fast-exchange cations, M^{n+}, based on line-width broadening of the water proton resonance when a small amount of paramagnetic metal ion is added. From this it is possible to determine the number of water molecules coordinated directly to H_4edta complexes of paramagnetic first row transition metal ions using measurements of the T_1 and T_2 times of the ^1H N.M.R. signal for bulk water. The values of the relaxation constants, T_1 and T_2, for this signal are easily obtained for such systems using Fourier Transform N.M.R. spectrometers with pre-programmed pulse sequences.

The system was calibrated, following the work of Oakes and Smith, using iron(III)/EDTA systems [2, 3]. Then a systematic study of iron(III)/IDA (IDA = iminodiacetic acid) systems was performed, and an interpretation of the results was made with reference to the known solid state chemistry of this iron-ligand system.

1 Burgess, J. *Metal Ions in Solution,* Chapter 2, 42-73, Ellis Horwood, Chichester, England, 1978.

2. Oakes, J., Smith, E.G., *J. Chem. Soc. Faraday Trans 2, 77,* 299-308, 1981:

3 Oakes, J., Smith, E.G., *J. Chem. Soc. Faraday Trans 1, 79,* 543-552, 1983

THE DIRECT ELECTROCHEMISTRY OF ENZYMES

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The *direct* electrochemistry of redox proteins is now well established whereas that of enzymes has only recently (1) been achieved. (The *direct* electrochemistry does *not* involve the transfer of electrons between electrode and enzyme *via* mediators.) Among those which have been reported include the electrochemistry of p-cresolmethylhydroxylase (2) and methylamine dehydrogenase (3). We have been interested in extending this work to include enzymes which may be of use in biosensors since there are obvious advantages in such systems. Consequently, we have investigated the electrochemistry of nitrate (4), nitrite (5,6) and nitrous oxide (7) oxido-reductases. More recently, we have studied (8) the electrochemistry of cytochrome P-450: interest centres on the behaviour of various genetic hybrids.

1. H. A. O. Hill and N. I. Hunt in J. F. Riordan and B. L. Vallee (Eds.), Metallobiochemistry, Part D, Methods in Enzymology Vol 227, 1993, 501.

2 L. H. Guo, H. A. O. Hill, D. J. Hopper, G. A. Lawrence and G. S. Sanghera, J. Electroanal. Chem., 266 (1989) 379.

3 A. L. Burrows, H. A. O. Hill, T. A. Leese, W. S. McIntire, H. Nakayama and G. S. Sanghera, Eur. J. Biochem., 199 (1991) 73.

4 B. C. Berks, S.J. Ferguson, H. A. O. Hill, N. I. Hunt and D. J. Richardson, to be published

5 R. Eady, H. A. O. Hill and P. M. A. Oliveira, to be published

6 H. A. O. Hill, N. I. Hunt, D. M. Keeley and P. Kroneck, to be published

7 H. A. O. Hill, N. I. Hunt, D. M. Keeley, P. Kroneck and D. M. A. NabiRahni, to be published

8 H. A. O. Hill, J. Kazlauskaite, L. L. Wong and A. C. G. Westlake, to be published

High-Potential Iron-Sulfur Protein (HiPIP) is Involved in Light Driven Electron Transport in *Rhodoferax fermentans*.

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A new high potential iron-sulfur protein (HiPIP) has been discovered, isolated and purified from the phototrophic bacterium *Rhodoferax fermentans*. The protein has a redox potential of +350 mV, and is a dimer in solution. The functional role of the HiPIP was investigated measuring the light-induced oxygen uptake mediated by the photosythetic reaction center (RC). HiPIP increased the rate of oxygen reduction by membranes from light-grown cells, in the presence of continuous light and exogenous reductant (ascorbate). The stimulus reached saturation at high concentrations of HiPIP, suggesting that a HiPIP-RC complex formed. Addition of antimycin A induced a further increase of oxygen reduction, indicating the absence of rate-limiting steps involving the quinol oxydase enzyme. These results suggest that HiPIP provides an electron transport pathway to the photo-oxydized RC, thus indicating a physiological role for HiPIP in *Rf. fermentans*.

Human Embryonic Haemoglobins.

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During the earliest stages of human development oxygen transport requires the participation of three embryonic haemoglobins. We have engineered separate yeast expression systems for each of these haemoglobins based on a plasmid construct containing the necessary two protein coding genes, each coupled to a synthetic galactose activated promoter. The equilibrium oxygen binding properties of each purified haemoglobin have been characterised, together with the modulation of these properties by [H+] and [2,3 D.P.G.]. These findings have been rationalised in terms of the presence or absence of functionally essential amino-acids in each case. Preliminary X-ray diffraction studies are in progress.

Denitrification enzymes of *Thiobacillus denitrificans*: Molecular and spectroscopic

properties

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Denitrification is a facultative way of anaerobic respiration. Each metabolic N,O-compound

can be used as terminal electron acceptor for membrane associated electron transport chains.

All reduction reactions are catalyzed by metal proteins containing multiple metal centres (1).

Organization of the required enzymes has been studied in various organisms. Comparison of

the denitrification enzymes leads to a common scheme in organization.

Herein we report on the purification and characterization of cytochrome cd_1-nitrite reductase

and copper nitrous oxide reductase from *Thiobacillus denitrificans*. Nitrite reductase and

nitrous oxide reductase activity are shown to be associated with the membrane. Nitrous oxide

reductase activity was inhibited by acetylene. Both denitrification enzymes were purified to

homogeneity after solubilization with Triton X-100. Cytochrome cd_1-nitrite reductase has been

purified and characterized previously (2,3). Its molecular weight was 66 kDa by SDS-PAGE.

N-terminal amino acid sequence comparison with *Pseudomonads* revealed highly conserved

motives. A native molecular weight of 160 kDa was determined for nitrous oxide reductase via

gel chromatography compared to 73 kDa by SDS-PAGE. At present, detailed spectroscopic

and biochemical investigations of the membrane-bound enzymes are in progress.

1) Zumft, W.G., Kroneck, P.M.H. (1990) Denitrification in soil and sediment p. 37-55

(Revsbech, N.P. and Sorensen, J., eds) Plenum Press, New York 1990

2) Sawhney, V., Nicholas, D.J.D. (1978) J. Gen. Microbiol. 106: 119-128

3) LeGall, J. et al. (1979) Res. Commun. 87: 355-362

Are metalloenzymes involved in the anaerobic reductive dechlorination ?

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Reductive dechlorination is the initial step in the anaerobic biodegradation of chlorinated compounds which form an important class of environmental pollutants. For the recalcitrant highly-chlorinated congeners it is the only possibility of biotransformation into harmless or aerobically degradable products. Reductive dechlorination of aliphatic compounds can be catalyzed by anaerobic bacteria in a cometabolic or respiratory process.

Tetrapyrrol cofactors such as iron(II) porphyrins, corrinoids, and factor F_{430} catalyze reductive dechlorination reactions in buffer with a reducing agent and are thought to be involved in cometabolic reductive dechlorination by methanogens, sulfate-reducers, homoacetogens, and others. For some of these bacteria the relationship between dechlorination activity and tetrapyrrol-containing enzymes was established. Methyl-coenzyme M reductase of methanogens, for example, reductively dechlorinates 1,2-dichloroethane to ethene and chloroethane and cytochrome P-450$_{CAM}$ functions in reductive dechlorination reactions catalyzed by *Pseudomonas putida* G786. Chlorinated ethanes and ethenes are transformed by two-electron reductions and subsequent protonation, β-elimination, or α-elimination to yield less chlorinated ethanes, ethenes, or carbene-derived products, respectively. Possible reaction mechanisms will be discussed.

The hypothesis that chlorinated compounds could be utilized as terminal electron acceptors in an anaerobic respiration process was postulated several years ago. Very recently, bacteria were indeed isolated which couple reductive dechlorination of an aliphatic compound to growth on a non-fermentable substrate such as dihydrogen or formate. *Dehalobacter restrictus* is one these strains. This bacterium is restricted to using tetrachloroethene as electron acceptor and dihydrogen as electron donor. Carbon is assimilated partially from organic compounds such as acetate and, if present, from carbon dioxide. Analysis of the 16S rRNA sequence revealed that *D. restrictus* belongs to a yet unknown group of bacteria. In cell-free extracts, dechlorinating activity can be shown by providing reduced methyl viologen as electron donor or mediator. The tetrachloroethene reductase seems to be located at the inside of the cell membrane, whereas the hydrogenase is on the outside. Whether or not the tetrachloroethene reductase is a metalloenzyme and the type of hydrogenase that *D. restrictus* contains is currently under investigation.

Solution and Coordination Chemistry of trans-a_2Pt^{II} with Cytosine Nucleobases

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With the antitumor activity of cis-$(NH_3)_2PtCl_2$ (Cisplatin) well established, a rich chemistry for this compound with nucleic acid constituents has been developed, including di- and multinuclear complexes with nucleobases. In contrast, an analogous chemistry based on the trans-a_2Pt^{II} isomer (a = NH_3 or amine) is not known to the same extent. During our studies with trans-$[a_2PtL_2]^{2+}$ (a = NH_3, CH_3NH_2; L = 1-methylcytosine, 1,5-dimethyl-cytosine) with metal ions we found a new type of heteronuclear complexes of the general structure 2 (M = Pd^{II} 2b, Hg^{II} 2c).[1,2] The poster deals with two aspects :

1a   1b   2
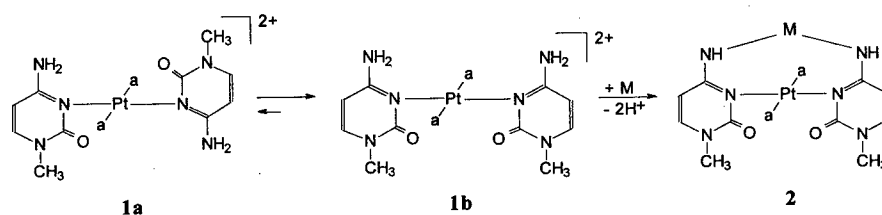
(1) In the heteronuclear complexes 2 the ligands are oriented head-head, whereas the starting compound 1 exists in solution as two rotamers, with head-tail (ht) 1a and head-head (hh) 1b oriented nucleobases in 4:1 ratio.[2,3] In the solid state, 1 exists as ht-rotamer only.[3,4] However, the hh-complex 1b can be isolated in high yield by removal of M (2) by means of a suitable nucleophile and rapid crystallization.

(2) We have been able to obtain novel heteronuclear derivatives. Starting from 1, the reaction with silver ions gives a trinuclear $PtAg_2$-complex 2c, in which the silver ions bind both to the deprotonated amino groups (N4) and the oxygens (O2) of the two bridging cytosine ligands. Another PtPd-complex 2b, containing the ligand N1-methylimidazol, has been also prepared. In this complex the imidazol nitrogen (N3) coordinates Pd^{II}.

1. M. Krumm, E. Zangrando, L. Randaccio, S. Menzer, B. Lippert, Inorg. Chem. 32 (1993) 700.

2. M. Krumm, E. Zangrando, L. Randaccio, S. Menzer, A. Danzmann, D. Holthenrich, B. Lippert, Inorg. Chem. 32 (1993) 2183.

3. G. Fusch, E.C. Fusch, B. Lippert, unpubl. results.

4. B. Lippert, C. J. L. Lock, R. A. Speranzini, Inorg. Chem. 20 (1981) 808.

The Type 2 Copper Centres of the Dissimilatory Nitrite Reductase from Alcaligenes xylosoxidans (NCIMB 11015) Bind the Substrate Nitrite

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We have recently shown that, contrary to earlier reports (1), the dissimilatory nitrite reductase (NiR) from *Alcaligenes xylosoxidans* subsp. *xylosoxidans* (NCIMB 11015) (Ax) is trimeric (2) and contains approximately equivalent levels of both Type 1 and Type 2 copper sites (3). We have now isolated a low-activity species of Ax NiR with essentially only Type 1 copper sites (4). We suggest that this is the species described by other workers (1). By utilizing both species we have been able to assign EPR and ^{1}H, ^{14,15}N ENDOR spectra of each type of copper site and to show that the substrate, nitrite, binds to the Type 2 copper site thus displacing a proton, probably on a water molecule bound to the copper atom. Further, our results show that the binding of nitrite significantly perturbs the ligation of the Type 2 copper by the protein histidine residues. Data on the binding of nitrite and the product nitric oxide to Ax NiR will be presented.

(1) Masuko, M., Iwasaki, H., Sakurai, T., Suzuki, S., and Nakahara, A. (1984) *J. Biochem. (Tokyo)* **96**, 447-454. (2) Grossman, J.G., Abraham, Z.H.L., Adman, E.T., Neu, M., Eady, R.R., Smith, B.E., and Hasnain, S.S. (1993) *Biochemistry* **32**, 7360-7366. (3) Abraham, Z.H.L., Lowe, D.J., and Smith, B.E. (1993) *Biochem. J.* **295**, 587-593. (4) Howes, B.D., Abraham, Z.H.L., Lowe, D.J., Brüser, T., Eady, R.R., and Smith, B.E. (1994) *Biochemistry* **33**, 3171-3177.

Magnetic and Magnetooptic Investigations on Cobalt-Substituted Hemocyanin-Derivatives

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A prevalent method to obtain further informations about the structure of the active site of metalloproteins consists in metal-substitution. Since Kitajima et al.[1] proposed a new model for oxyhemocyanin, where the two Cu(II)-centers show a $\mu-\eta^2:\eta^2$-peroxo-bridging structure, the question of the bridging ligand and binding situation in the native protein and its derivatives is still open.

Our temperature dependent magnetic susceptibility measurements show strong spin-coupling between binuclear Co-substituted Hc-Derivatives of the arthropod Carcinus maenas. The coupling constant is influenced by the variation of the bridging ligand. The binuclear Co(II) active site of Carcinus maenas-Hc can be characterized as a distorted tetrahedral antiferromagnetically coupled system, which is consistent with EPR-measurements[2] and earlier results concerning Limulus-Hc[3,4]. Compared to the binuclear derivatives, the mononuclear Co(II)-derivative shows reduced magnetic moments. This is consistent with a geometry which lies between tetrahedral and square planar arrangement[2].

Studies of the magnetic circular dichroism indicate tetracoordinate high-spin Co(II) with differences in the geometries of the binuclear-, the mononuclear mono-fast-, and the hybrid-derivative, which contains one Co(II)- and one Cu(I)-ion per active site.

1) N. Kitajima, K. Fujisawa, Y. Moro-oka and K. Toriumi, J. Am. Chem. Soc. 1989, **111**, 8975.

2) L. Bubacco, R.S. Magliozzo, M. Beltramini, B. Salvato, and J. Peisach, Biochem., 1992, **31**, 9294.

3) J. Lorösch and W. Haase, Biochem., 1986, **25**, 5850.

4) T. J. Dutton, T. F. Baumann, and J. A. Larrabee, Inorg. Chem., 1990, **29**, 2272.

ENDOR of Iron-Sulfur-Proteins

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ENDOR (Electron Nuclear Double Resonance)-Spectroscopy has since long proven to be a powerful tool in the determination of nature of ligands to the metal-ion in metalloproteins. More recently, tools were developed for determining spatial arrangement of protons (and, if available, ^{14}N, ^{15}N, ^{57}Fe nuclei) from ENDOR of randomly oriented model compounds[1] and metalloproteins[2] in systems with S = 1/2. We have extended this approach to (Fe_2S_2) and (Fe_4S_4) iron-sulfur clusters and present data for the following proteins: Chinolin-Oxidoreductase, Enoate Reductase, Halophila HIPIPII. Combining, whenever available, data about spin-density distributions in model compounds[3], isotropic couplings from NMR hyperfine shifts[4] and crystal structure data it will be shown that, for all cases the reduced or oxidized FeS-cluster ENDOR-data can be simulated with good agreement thereby allowing for the spatial construction of the proton-positions in the cluster.

1. Hurst, G.C.; Henderson, T.A.; Kreilick, R.W.; J. Am. Chem. Soc., 1985, 107, 7294.
   Däges, G.P.; Hüttermann, J.; J. Phys. Chem., 1992, 96, 4787.
   Scholl, H.-J.; Hüttermann, J.; J. Phys. Chem., 1992, 96, 9684.
2. Hüttermann, J.; Burgard, C.; Kappl, R.; J. Chem. Soc. Faraday Transactions, 1994, in press.
   Reinhard, H.; Kappl, R.; Hüttermann, J.; Viezzoli, M.S.; J. Phys. Chem., 1994, in press.
3. Rius, G.; Lamotte, B.; J. Am. Chem. Soc., 1989, 111, 2464.
   Mouesca, J.-M.; Rius, G.; Lamotte, B.; J. Am. Chem. Soc, 1993, 115, 4714.
4. Bertini, I.; Briganti, F., Luchinat, C.; Scozzafava, A.; Sola, M.; J. Am. Chem. Soc., 1991, 113, 1237.
   Bertini, I.; Capozzi, F.; Ciurli, S.; Luchinat, C.; Messori, L.; Sola, M.; J. Am. Chem. Soc., 1992, 114, 3332.

Preliminary studies on the vanadium haloperoxidases from the fungi *Mycosphaerella ascophylli.*

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Vanadium haloperoxidases can be found in one lichen, two terrestrial fungi [1,2] and in red, green and brown algae, namely in *Ascophyllum nodosum*. In the terrestrial fungi *Curvularia inequalis* [3] it has been found that an inactive haloperoxidase is excreted in the culture media only when the nutrients are near to exhaustion; to recover activity vanadate must be added.

Ascophyllum nodosum possesses an endophytic mycophycobiosis [4], with the fungus *Mycosphaerella ascophylli*, making it similar to a lichen. In order to clarify which of the organisms *Ascophyllum nodosum* or *Mycosphaerella ascophylli* is responsible for the synthesis of the vanadium haloperoxidases the fungus was isolated from the alga and cultivated in axenic agar culture [5,6]. When the cultivated fungi reached the size of a small coin, samples of the solid culture media were transferred to liquid culture media containing 0, 4 and 40 mg of vanadate. In solutions containing 0 and 4 mg of vanadium, *Mycosphaerella ascophylli* grew (more in 0 mg than 4 mg of vanadium) but not in the solution containing 40 mg of vanadium. Optical microscopic observations revealed that the fungus was dead at this vanadium concentration, showing the toxic effect of this trace element at higher concentrations. However, the culture medium exhibits haloperoxidase activity and it has been found that there is a direct correlation between this activity and the total concentration of protein excreted by the fungus. The activity and the amount of protein increase with the amount of vanadium in the medium and are highest for the samples containing 40 mg of vanadium.

Comparision of the haloperoxidases from the fungus and of the two haloperoxidases isolated by Wever *et al* [7] and in other species of *Ascophyllum nodosum* are being undertaken to trace their possible origins.

DNA extraction from the alga and the fungi are also being carried out with the same objective. So far this has been sucessful for the algae's DNA.

[1]- van Schijndel, J.W.P.M., Vollenbroek, E.G.M., Wever, R. *Biochim. Biophy. Acta* (1993), **1161**, 249

[2]- Wever, R., Krenn, B.E., de Boer, E. Eur. J. Biochem. (1990), **187**, 205-211

[3]- "The biology of marine fungi", D.L Kingman, L.V. Evans, S.T.Moss eds. Cambridge Univ. Press , Cambridge, 1986

[4] - Kohlmeyer & Kohlmeyer Bot. Mar.(1972), **15**, 109-112

[5]- Fries, N. Physiol. Plant (1979), **45**, 111-121

[6]- Fries, C. *J. Phycol* (1988), **24**, 333-337

[7]- Krenn, B.E., Tromp, M., Wever, R. J. Biol. Chem (1989), **264**, 19287-19292

IRON COMPLEXES OF TRIDENTATE LIGANDS WITH IMIDAZOLE AND AMINE DONOR GROUPS: STRUCTURAL, ELECTRONIC AND MAGNETIC STUDIES.

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The interaction of iron with histidyl residues is a frequent phenomenon in iron containing proteins. Examples include the reaction center of Photosystem II[1], lipoxygenase[2], or isopenicillin N synthase[3]. Related molecular models are of interest to assess the influence of the imidazole ring on the spectroscopic properties of the iron active site.

We have synthesized and characterized by X-rays a series of ferrous complexes with the tridentate ligand (N-methylimidazole-$CH_2)_2NR$ (R= H, CH_3). Their electronic, magnetic and redox properties will be presented and compared to our results with {Fe_2S_2[(N - methylimidazole)$_2CHOCH_3]_2Cl\}_n$ (n=1 or 2). In particular, the ^{1}H-NMR resonances appear to be very sensitive to the magnetic and oxidation state of the iron, whereas the Mössbauer parameters are strongly influenced by anion-dependent lattice effects.

1. Okamura, M.Y., Feher, G. Annu. Rev. Biochem. (1992) 61, 861-896.
2. Navaratnam, S., Feiters, M.C., Al-Hakim, M., Allen, J.C., Veldink, G.A., Vliegenthart, J.F.G. Biochim. Biophys. Acta (1988) 956, 70-76.
3. Ming, L.J., Que, L., Frolik, C., Krauciunas, A., Chen, V. J. Inorg. Biochem. (1989) 36, 264.

Investigation of the role of a conserved tyrosine in a High Potential Iron Sulfur Protein

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Tyrosine 12, a conserved residue in the 4Fe-4S cluster binding pocket of the iso-I high potential iron sulphur protein (HiPIP) of *Ectothiorhodospira halophila* was sustituted with alanine, isoleucine, histidine, phenylalanine and tryptophan by oligonucleotide-directed mutagenesis. The alanine and isoleucine variants (Y12A and Y12I, respectively) were expressed at reasonable levels in cultures grown at 25 °C, but decomposed during the course of the purification. The phenylalanine, tryptophan and histidine variants (Y12F, Y12W and Y12H, respectively) were stable during expression at 37 °C and were purified and further characterized. CD and NMR spectroscopic analyses indicate that the environment around the cluster of Y12F is very similar to that of wild-type HiPIP. The reduction potential of Y12F was determined spectroelectrochemically to be 134.1 mV (20 mM HEPES, 80 mM NaCl, pH 7.0, at 25 °C) versus the standard hydrogen electrode (S.H.E.) which is approximately 14 mV higher than that of wild-type HiPIP iso-I of *E. halophila*. Spectroscopic and electrochemical data of Y12H and Y12W will be presented. The observations indicate that the residue at position 12 plays a major role in mediating cluster structure and function. More specifically, the instability of the HiPIP variants possessing a non-aromatic residue at position 12 suggests that the cluster is stabilized by a π–π interaction between tyrosine-12 and the adjacent sulfur atom of the [4Fe-4S] cluster.

Aquation and DNA platination mechanism
of substituted ethylenediaminedichloroplatinum(II) complexes
with very different *in vivo* antitumor activities

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Diaminedichloroplatinum(II) complexes with bulky more or less hydrophobic bicycloalkyl substituents on the ethylene diamine ligand were synthesized and their *in vivo* biological activities were found to be very different (1). As DNA is believed to be an important biological target of cisplatin (2), the reaction of these complexes with DNA was investigated.

The rate limiting step of DNA platination by cisplatin is the first aquation reaction (3). The aquation reaction has been studied using UV-visible spectrophotometry as a function of pH, of reactant concentration and of temperature.
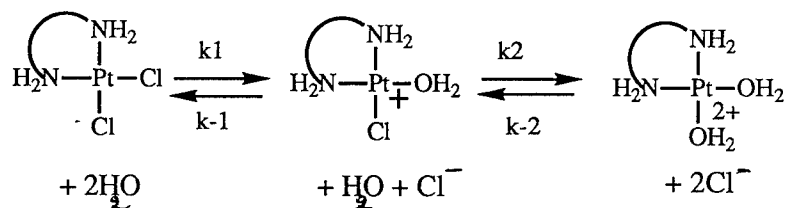
The pressure dependence of the kinetics allowed the determination of the whole volume profile in cm³/mol.: $\Delta V^{\neq}_1$=-9.4, $\Delta V^{\neq}_{-1}$=-4.0, $\Delta V^{\neq}_2$=-6.6, $\Delta V^{\neq}_{-2}$=-4.4. These activation volumes are characteristic of an associative mechanism (4).

The DNA platination kinetics was established using UV-visible spectrophotometry and atomic absorption spectroscopy and studied as a function of DNA concentration in pseudo-first order conditions. A two-step reaction mechanism involving a first aquation step and a DNA platination step by the monochloromonoaquacomplex is consistent with the results. The first and second-order rate constants calculated are compared to those obtained for cis-DDP.

A possible relationship between the nature of the substituted ethylenediamine and the *in vivo* antitumor activity will be discussed.

References:
(1)-Mailliet,P., Segal,E., Barreau,M., Baudouin,B., Gontier,S., Kozelka,J;, Laoui,A., Lavelle,F., Le Pecq,J-B., Chottard,J-C. *Anti-Cancer Drug Design, submitted for publication.*
(2)-Bruhn,S.L., Toney,J.H., Lippard,S.J. *Progress in inorganic chemistry,* **1990,** 477-516.
(3)-Johnson,N.P., Hoeschele,J.D., Rahn,R.O. *Chem-Biol. Interactions,* **1980,** *30,* 151-169.
(4)-Jestin,J-L., Laurenczy,G., Chottard, J-C, Merbach,A.E. *Inorg. Chem., submitted for publication.*

ANHYDROSUGARS - UNEXPECTEDLY MANIFOLD LIGANDS TOWARDS COPPER(II).

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Significance of the 1,6-anhydro-amino acids in the bioinorganic chemistry results from presence in their molecule the amino group and dioxolene ring. Both of them are found in natural antibiotics produced by funguses and their synthetic derivatives. The binding pattern of 1,6-anhydro-amino acid with Cu(II) ion (Cu(II) - the endogenic factors of human immunity) is potentially important for complete description of the interaction mechanism and antibiotics toxicity.[1,2]

Our pH-metric, UV-VIS, C.D. and EPR study has shown that these ligands binds Cu(II) in a bidentate manner. The chelate ring contains the amino nitrogen and suitable hydroxyl oxygen donor. Configuration and conformation of ligands affect critically complex stabilities and in some cases can promote the formation of dimeric species, contained six-membered chelate rings with bridging -OH groups. The dimer formation was supported by EXFAS study too[3]. The dimer formation occures in the case of 2- and 4-amino derivatives, where the stabilization of chelate ring by 1,3-dioxolene ring is likely. This is not the case for the 3-amino derivatives. Deoxy O-alkil, O-aryl and 3,4-epimino derivatives are also unable to form dimers.

1. M.Jezowska -Bojczuk, H.Kozlowski, P.Decock, ,T.Trnka ,M.Cerny, *Carbohydr.Res.*, **216** (1991), 453.
2. M.Jezowska -Bojczuk, H.Kozlowski, T.Trnka, M.Cerny, *Carbohydr.Res.* ,**253,** (1994), 19.
3. L.Nagy, T.Yamaguchi, T.Mitsunaga, H.Wakita, M.Jezowska-Bojczuk, H.Kozlowski, *Inorg. Chim.Acta in press.*

THE CHARACTERISATION OF ALUMINIUM IN BIOLOGICAL SYSTEMS

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Transferrin is the major iron transport protein in blood. It has been shown that *in vitro* transferrin will bind a wide range of metals including Ga^{3+}, Al^{3+}, Zn^{2+}, with lower binding affinities than for Fe^{3+}. Given that *in vivo* only 40% of transferrin has iron bound there is a great potential for the protein to transport other metals. Aluminium uptake into cells appears to involve it being bound by transferrin. Although the most abundant metal in nature, Al^{3+} has no known beneficial biological function in animals[1]. It has been implicated in a variety of medical disorders in humans[2] and has been shown to be toxic to fish. However many of the factors governing the uptake and distribution of Al^{3+} within the body remain unknown. Metabolism will not be confined to proteins but will also involve interaction with small molecules. Therefore it is necessary to study the speciation of Al^{3+} in systems containing both proteins and small molecules. A key feature of such speciation studies is that they should closely reflect physiological conditions.

I have studied the pH dependency of Al^{3+} binding to serum transferrin. There is a striking difference between Al^{3+} and Fe^{3+} binding under acidic conditions. The affinity for Al^{3+} drops sharply at pH <7.4 but that for Fe^{3+} remains stable until pH <6.5, which may be why Al^{3+} is excreted via the kidney while iron is retained. The characterisation of Al^{3+} with small molecules is more difficult than studying its interaction with proteins due to the tendency of the metal to hydrolyse and the lack of an easy spectroscopic probe. We can now address these questions of speciation using a combination of solution and solid state studies. The main techniques involved are X-Ray crystallography, solution and solid state multinuclear NMR spectroscopy, potentiometry, and vibrational spectroscopy.

This approach is illustrated by the interaction of Al^{3+} with the ligand HEIDI $[N(CH_2CO_2H)_2(CH_2CH_2OH)]$, which is the best characterised system to date. This procedure can now be applied to physiological systems like citrate or malate. Crystallisation trials have led to the characterisation of two solid state structures. With an excess of HEIDI over Al^{3+} the dinuclear compound $[Al(HEIDI)(H_2O)]_2 \cdot 2H_2O$ can be isolated. In corresponding solutions with an excess of Al^{3+} it is possible to isolate a 13-aluminium cluster $[Al_{13}(\mu_3\text{-}OH)_6(\mu_2\text{-}OH)_{12}(HEIDI)_6(H_2O)_6]^{3+}$. The parallel use of solution and solid state NMR serves as a link between solid and solution phases. This assists the determination of solution speciation and highlights the thermodynamic relevance of the solid species. It is now possible to define the presence of 6 aluminium species in this system.

References:
1. *Aluminium in Chemistry,Biology and Medicine*
   (Eds. M. Nicolini, P.F. Zatta, B. Corain), Cotina International, Verona, 1991.
2. *Aluminium in Biology and Medicine* : CIBA Foundation Symposium 169
   (Eds. D.F. Chadwick, J. Whelan), Wiley-Interscience, Chichester, 1992

The structure of *Pseudomonas stutzeri* cytochrome c_4 studied by NMR spectroscopy and X-ray diffraction.

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Cytochrome c_4 (MW $\approx$ 20 kD, 190 amino acid residues) has been isolated from *Pseudomonas stutzeri, Azotobacter vinelandii, Pseudomonas aeruginosa* and *Alcaligenes sp.* The function of cytochrome c_4 is unknown.

The crystal structure for *Pseudomonas stutzeri* (A.T.C.C. 11607) cytochrome c_4 has been obtained. It shows cytochrome c_4 as being a two-domaine, di-heme protein with histidine and methionine as axial ligands for both hemes. The propionate groups from each heme are oriented towards each other and are within hydrogen bonding distance. The hemes are not co-planar. It also shows a hydrogen-bond network in the vicinity of the hemes and in the region between the two domains. From the distribution of surface charges it is apparent that cytochrome c_4 is a dipolar protein. Parameters from the crystal structure can be used in electrostatic models to estimate the static cooperativity in cytochrome c_4 which is due to the electrostatic potential aroused by localized electric charges inside or outside the protein.

The NMR spectrum of the reduced form of cytochrome c_4 is typical of a low spin cytochrome c. The identification of the meso- and thioether protons and of the heme- and thioether methyl groups of each heme was achieved using 2D NOESY and TOCSY results obtained in the reduced state (strategy as in ref.[1]). A study of the temperature dependence in the reduced state showed no significant changes in shifts thus indicating that both hemes are low spin in the reduced state.

The NMR spectrum of oxidized cytochrome c_4 is similar to that from *Azotobacter vinelandii* showing resonances down to app. 50 ppm[2]. These shifts are abnormally high for pure low spin proteins. A study of the temperature dependence of oxidized cytochrome c_4 showed that most low field resonances had anti-Curie behaviour. These results may be explained by a temperature dependent equilibrium between a low spin and a high spin form.

Ps. stutzeri cytochrome c_4 has also been characterized by EPR and low temperature visible absorption spectroscopy. The low temperature spectrum shows α-band splitting and EPR shows two distinct heme groups that are mainly low spin at 4 K.

[1]Coutinho, I. B.; Turner, D. L.; Legall, J. and Xavier, A. V., Eur. J. Biochem., 209, 329-333, 1992.
[2]Gadsby, P. M. A.; Hartshorn, R. T.; Moura, J. J. G.; Sinclair-Day, J. D.; Sykes, A. G. and Thomson, A. J., Biochim. Biophys. Acta, 994, 37-46, 1989.

THE INTERACTION OF GROUP VIIIA METAL IONS WITH DNA

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The effect of metal ions upon conformation of DNA was
realized early, when it became apparent that metal ions are
involved in the stabilization of the Watson-Crick double helix.
Metal ions react with a variety of electron-donor sites on poly-
nucleotides. There are two main sites of interaction, the
phosphate moieties of the ribose-phosphate backbone and the
electron-donor groups of the bases. The two types of interaction
carry with them quite different effects upon the structure of
polynucleotides. Reactions with the phosphate means stabilization
of ordered structures but cleavage of phosphodiester bonds at
high temperature. Whether the binding is non-specific, i.e.,
totally predictable on the basis of electrolyte theory, or whether
specific bonds to the phosphate are produced, the result is to
neutralize the array of negative charges on the double helix
and thus to stabilize it. Such stabilization is accompanied
by an increase in the "melting temperature" of DNA.

Reaction with the bases means destabilization of ordered
structures, since metal ions can bind to the base stacking inter-
actions that hold together the two strands of DNA. Destabilization
is accompanied by a decrease in the melting tempetature, T_m of
DNA. The differences in behavior of various metal ions with
polynucleotides have made it apparent that some metal ions prefer
the phosphate sites and other metal ions prefer the base sites.
The difference was strikingly illustrated by the effect of mag-
nesium (II) and cadmium (II) ions on the melting behavior of
DNA. However, metal ions cannot be placed into two categories
of those that bind to phosphate and those bind to the bases.
Thus, copper (II) ions that are so effective in base binding,
bind to also phosphate and are therefore capable of cleaving
phosphodiester links in polyribonucleotides. On the other hand,
zinc (II) ions which are so effective in degrading phosphodiester
links due to phosphate binding have been demonstrated to bring
about a temperature-reversible unwinding of DNA through binding
to the bases.

Several ruthenium complexes have been shown to inhibit
cellular DNA synthesis in vitro at a level similar to that of
cis-Pt(NH$_3$)$_2$Cl$_2$ and have shown antitumor activity in animal
studies. Compounds containing pentammine-ruthenium(II) and
(III) have been shown to inhibit DNA synthesis functioning,
probably by binding to DNA. The interaction and conformational
changes of various heavy metal ions among them Ru(II), Ru(III), Rh(II),
Rh (III) systems with DNA is presented.

Mummified Zn$_2$Mg alkaline phosphatase

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Our knowledge to the mode of conservation of mummified structurally and functionally intact biopolymers is limited. Rib samples of a well preserved 2300 year old ptolemeic mummy were examined whether or not functionally active Zn$_2$Mg alkaline phosphatase could be detected. A protein of M$_r$ 170 $\pm$ 20 kD being close to 200 kD of the enzyme of fresh bones was successfully isolated. Both a 200 kD protein and a distinct subunit of 60 kD were seen in SDS-page electrophoresis which was identical to those of fresh bone alkaline phosphatase. There was a significant enzymic activity of 17 mU/mg protein which could be inhibited in the presence of L-homoarginine and 1,10-phenanthroline.

METALLACYCLES WITH NUCLEOPHILIC LIGANDS AS ENZYME MODELS

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Cyclometallated complexes of Pd(II) and Pt(II) with nucleophilic ligands could be considered as models for various metalloenzymes such as carbonic anhydrase, carboxypeptidase A, piruvatkinase etc. The catalityc activity of these complexes in the ester and phosphate hydrolysis is based on the same principles as enzymatic catalysis — generation of the strong nucleophilic center in the low pH media. The effective activity of the hydroxide ion coordinated in trans—position to the metal—carbon σ—bond in the 2,4—dinitrophenyl acetate hydrolysis is 10^{10} times higher than that of the noncoordinated one. As a result of coordination, its basicity increases more than by 11 orders of magnitude, but the reactivity by a factor of only 100. Cyclometallated aryl oximes also catalyze the ester hydrolysis and the same catalytic effect is realized. Their reactivity at neutral pH is 10^4—fold higher than for the nonmetallated ones. Because of cyclometallation aryl oximes become true catalysts since a catastrophic acceleration (more than 10 orders of magnitude) of deacylation of the O—acyl oxime intermediate takes place. In the same manner the complexes mentioned above catalyze hydrolysis of the 2,4—dinitrophenyldiethyl phosphate.

As in the case of enzyme catalysis, the assembling of the substrate and nucleophile to the one complex leads to dramatic increase in the reaction rate: the hydrolysis of the S—coordinated with Pt and Pd t—BOC—methionine esters in the complexes with ortho—metallated ligand and hydroxo ligand proceeds much faster not only for noncatalytyc reaction but also for intermolecularly catalyzed process.

ELECTRON TRANSFER AND HEME-HEME COUPLING IN CYTOCHROMES C3.

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The intramolecular electron transfer in tetrahemic cytochromes c_3 from *Desulfovibrio desulfuricans* Norway, and *Desulfovibrio vulgaris* Hildenborough has been studied by thin layer spectroelectrochemistry. The U.V.-Visible absorption spectra, generated by stepwise variation of the imposed potential display 2 distinct groups of isosbestic points: the first group encompasses reduction ratios 0-25% and 0-50% for cyt.c_3 Hildenborough and cyt.c_3 Norway respectively, whereas the second group corresponds to reduction ratios up to 100%. Therefore the redox process can be schematically written:

for cyt.c_3 Hildenborough

$$(Fe^{III})_4 \longleftrightarrow (Fe^{III})_3 Fe^{II} \longleftrightarrow (Fe^{II})_4$$

for cyt.c_3 Norway

$$(Fe^{III})_4 \longleftrightarrow (Fe^{III})_2 (Fe^{II})_2 \longleftrightarrow (Fe^{II})_4$$
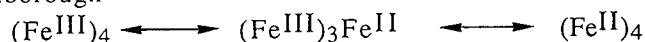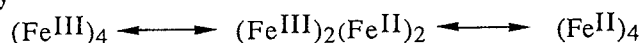
A comparable scheme has been recently proposed for cyt. c_3 Norway, by D. Schlereth et al.(1).

Circular Dichroïsm and Resonance Raman spectra of the intermediate redox state have been obtained, for both cytochromes, and compared to the spectra calculated for the same reduction ratio from the fully oxidized $(Fe^{III})_4$ and fully reduced $(Fe^{II})_4$ species. The differences thus revealed will be discussed. Most noticeable, in the case of cyt.c_3 Norway, a unique intense Soret CD signal has been observed for the intermediate redox state.

1. D. D. Schlereth, V. M. Fernandez, and W. Mäntele, Biochem. 32, 9199-9208 (1993)

SPECTROSCOPIC STUDIES OF DIVALENT METAL SUBSTITUTED INSULIN.

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Insulin, a monomeric polypeptide hormone will aggregate in the presence of divalent metal ions to form hexamers with the general formular $M(II)_2$ Ins_6. Insulin is stored in the pancreatic islets as the hexamer $Zn(II)_2Ins_6$.

Zn may be substituted with other divalent metal ions. These species possess octahedral coordination comprising 3 His and 3 H_2O.

The addition of phenol to these insulin species produces an allosteric change (1). Each monomer binds a single phenol molecule and undergoes a transition designated T to R whereupon the B chain residues adopt a helical conformation. This drives a change in coordination geometry for the metal sites from octahedral to tetrahedral with three endogenous His ligands and either H_2O or an anion from solution as the fourth ligand. This site provides the potential to model various metalloproteins by varying both the fourth ligand and metal.

In this work Co (II) (2), Cu (II) and Fe (II) substituted insulins have been studied. The binding of these metal ions to T and R state insulin and the subsequent addition of exogenous ligands have been followed by UV/visible and Magnetic Circular Dichroism spectroscopies.

References

(1)   Dodson G. et al. Nature. **338**, (1988) *594-596*.
(2)   Dunn M.F. et al. J. Biol. Chem. **264**, <u>32</u>, (1989) *19081-19085*.

MANGANESE CONTAINING BIOLOGICAL SYSTEMS AND RELEVANT TRINUCLEAR SYNTHETIC COMPLEXES

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Today it is known that Manganese is an essential element in many biological processes e.g. Oxygen Evolving Complex of Photosystem II, Superoxide Dismutase etc. Two functional values can be distinguished, first Mn^{2+} as a Lewis acid and second in higher oxidation states(Mn^{3+}, Mn^{4+}) as an oxidation catalyst. In the most well known manganoproteins the role of Mn^{2+} ions has not firmly established e.g. *Arginase, Concanavalin A, Glutamine Synthetase etc.* For the enzymes containing high oxidation state manganese ions, the most characteristic function is catalytic e.g. for the Oxygen Evolving Complex(OEC) in Photosystem II, has been well established that manganese involves in the reaction center. The OEC contains four manganese ions.

Relevant Trinuclear Synthetic Complexes. The first open structure trinuclear complex was the mixed valence compound α-Mn(II)/Mn(III)$_2$(saladhp)$_2$(OAc)$_4$ (CH$_3$OH)$_2$. Water can be substituted for methanol to give the isostructural α-Mn(II)/Mn(III)$_2$(saladhp)$_2$ (OAc)$_4$(H$_2$O)$_2$. The neutral axial ligand can be substituted for 2-hydroxypyridine or dmf to generate the linear α-Mn(II)/Mn(III)$_2$(saladhp)$_2$ (OAc)$_4$(HpyrO)$_2$ and α-Mn(II)/Mn(III)$_2$(5-Cl-saladhp)$_2$(OAc)$_2$(5-Cl-salac)$_2$(dmf)$_2$ respectively.

References

1. Pecoraro,V.L.,*Manganese Redox Enzymes,* VCH Publishers Inc, New York, **1992**

2. Kessissoglou, D.P. ; Kirk, M.L.;Lah,M.S.; Li,X.h.; Raptopoulou, C.A.; Hatfield, W.E.; Pecoraro, V.L. , *Inorg. Chem.*, **1992**,*31*,5424;

GLOBAL ANALYSIS OF TIME DEPENDANT SPECTRA

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The use of stopped-flow absorption and fluorescence spectroscopy to determine reaction mechanisms and rate constants in biochemical systems has long been established. Traditional non-linear least squares analysis of a kinetic trace can routinely determine the number and magnitude of exponential components in the reaction. More recently the introduction of instruments which generate high quality kinetic data at multiple wavelengths have given the kineticist the potential to establish these reaction parameters with greater precision and the opportunity to analyse reactions of far greater complexity. However the size of the data matrix is great, requiring a large amount of computer memory for storage and placing much more emphasis on data handling and analysis.

We report on the use of **Single Value Decomposition** as a means of reducing the size of data sets as well as providing a model free assessment of the minimum complexity of the reaction mechanism prior to analysis. And on the use of **global analysis with numerical integration** to determine rate constants and intermediate spectra based upon virtually any proposed reaction mechanism.

Copper-containing Oxidases: enzymes with novel built in cofactors.

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Abstract

There are a growing number of examples of enzymes which use modified polypeptide chain amino-acid residues as cofactors. These include quinone derivatives such as tryptophane tryptophyl quinone (TTQ) found in methylamine dehydrogenase and tyrosyl radicals found in ribonucleotide reductase. Copper-containing oxidases use both quinones and radicals in their catalytic mechanisms: amine oxidases have trihydroxyphenylalanine (TOPA) as cofactor whilst galactose oxidase has a tyrosine free radical stabilised through a novel thio-ether bridge and a stacking tryptophane in its active site. Our studies using X-ray crystallography, protein engineering, spectroscopy and kinetics are directed towards elucidating the molecular basis for catalysis in copper-containing oxidases; we are also using molecular biological approaches to understand the role of these enzymes in cellular regulation.

References

(1) Ito N., Phillips S.E.V., Yadav K.D.S. and Knowles P.F. (1994) J. Mol. Biol. 238, 794-814.

(2) McPherson M.J, Ogel Z.B., Stevens C., Yadav K.D.S., Keen J.N. and Knowles P.F. (1992) J.Biol.Chem. 267, 8146-8152.

(3) Dooley D.M., McGuirl M.A., Brown D.E., Turowski P.N., McIntire W.S. and Knowles P.F. (1991) Nature 349, 262-264.

Iron Catecholate Complexes as Functional Models for Intradiol-Catechol-dioxygenases

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Catecholdioxygenases are non-heme iron-containing enzymes catalyzing the oxidative cleavage of the *intradiol* C-C bond of catecholate to yield muconic acid. The active site consists of a ferric ion coordinated to two histidines, two tyrosinates and a water molecule in a trigonal bipyramidal geometry.[1] The most unusual property of this iron centre is that the ferric oxidation state is retained during the whole catalytic cycle.

As part of our efforts to obtain functional models for these enzymes, ferric catecholate complexes were synthesized with a tetraazamacrocyclic ligand (L). The structure of the iron catecholate complex is shown in Figure 1.
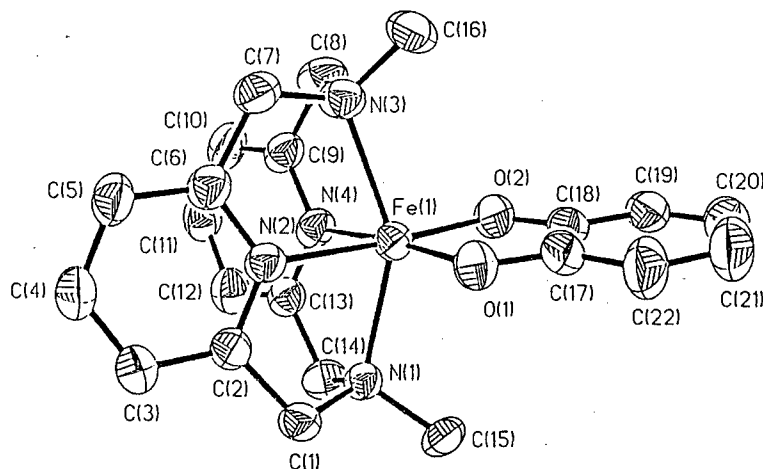
Figure 1: Structure of the $[(L)Fe(O_2C_6H_4)]^+$

Reactivity studies demonstrated that iron bound 3,5-di-t.-butylcatecholate was quantitatively converted to the corresponding muconic acid by oxygen. The electronic properties of the functional models are compared to those of the enzyme and discussed with respect to the reaction mechanism. The role of a bound semiquinone in the reaction mechanism will be examined.

(1)   Que, L., Jr. in Iron Carriers and Iron Proteins (ed. Loehr, T.M.), VCH, Weinheim, **1989**, p. 467.

Reaction Chemistry of Dinuclear Iron Benzoate Complexes as Model for the Oxidative Conversion of the Reduced Iron Center in Ribonucleotide Reductase

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Iron-containing Ribonucleotide Reductases are members of a class of metalloproteins in which the active sites consist of non-heme dinuclear iron clusters. Crystallographic data[1] of the Mn(II)-substituted enzyme imply that the reduced form consists of two Fe(II) ions bridged by two carboxylates. Upon oxidation with molecular oxygen the iron site is converted to a μ-oxo-μ-carboxylato bridged diferric species.[2]

The starting point of our investigation represents the bis(μ-benzoato-O,O´)diiron(II) complex (Figure 1) whose remaining coordination sites are occupied by the tetraazamacrocyclic ligand L.
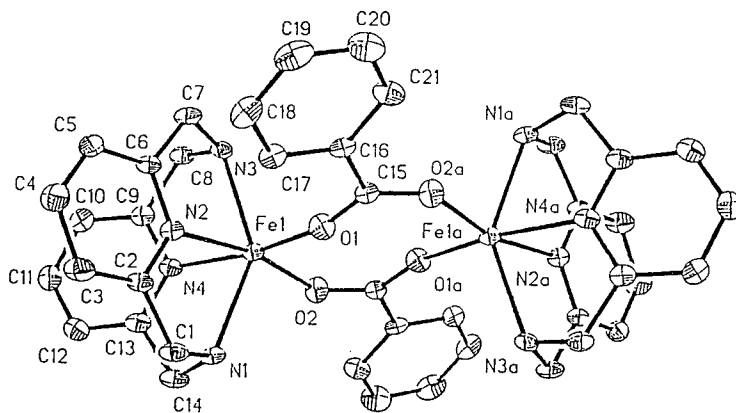
Figure 1: Structure of $[(L)Fe(\mu\text{-}O_2CPh)_2Fe(L)]^{2+}$

Upon reaction with molecular oxygen the diferrous complex $[(L)Fe(\mu\text{-}O_2CPh)_2Fe(L)]^{2+}$ forms a very stable singly-bridged diferric complex $[(L)(PhCO_2)Fe(\mu\text{-}O)Fe(O_2CPh)(L)]^{2+}$. By treatment with acid this complex reacts to the μ-oxo-μ-benzoato-bridged diferric species $[(L)Fe(\mu\text{-}O)(\mu\text{-}O_2CPh)Fe(L)]^{3+}$. All described complexes are thoroughly characterized by various spectroscopic means.

(1)   Atta, M.; Nordlund, P.; Åberg, A.; Eklund, H.; Fontecave, M. *J. Biol. Chem.* **1992**, *267*, 20682.

(2)   Nordlund, P.; Eklund, H.; Sjöberg, B-M. *Nature* **1990**, *345*, 593.

Abstract of the invited session lecture

Organometallic titanium compounds: antitumor agents in preclinical and early clinical trials

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About fifteen years ago, the antitumor activity of titanocene dichloride $[(C_5H_5)_2TiCl_2]$, the main representative of antitumor organometallic titanium compounds, was described first. In the meanwhile, the antitumor activity of titanocene dichloride was confirmed against numerous experimental animal and human carcinomas in vivo and in vitro, and the toxicologic and pharmacokinetic characteristics were defined at the preclinical level. In December 1991, titanocene dichloride was introduced into phase I clinical studies which aim at the determination of the dose-limiting toxicity and the appointment of the maximum tolerated dose (MTD) in human patients. Until April 1994, the dose of titanocene dichloride applied intravenously as a single injection to human patients could be escalated up to 560 mg/m^2 corresponding to 14.5 mg/kg, without reaching the MTD. The main toxic symptoms observed so far in human patients were nephrotoxicity, gastrointestinal irritations including the dose-dependent occurrence of a metallic taste immediately after treatment, and sporadic, reversible depressions of the blood glucose level.

Other organometallic titanium complexes such as titanocene acetonitrile tetrachloroferrate $[(C_5H_5)_2TiCl(NCCH_3)]^+(FeCl_4)^-$, are investigated presently as back-up compounds of titanocene dichloride. They are characterized by similar patterns of antitumor and toxicologic properties as titanocene dichloride at the preclinical stage.

Recently performed investigations into the molecular mode of action of titanocene dichloride revealed direct titanium-DNA and titanium-amino acid interactions and confirmed the formation of stable adducts of bis(cyclopentadienyl)titanium(IV) and mono(cyclopentadienyl)titanium(IV) moieties both to DNA and amino acid molecules. The mode of binding clearly argued against cisplatin-like complexation motifs of antitumor titanocene complexes.

These results approve organometallic titanocene complexes to be prospective antitumor agents investigated currently in early clinical studies. They lack experimental cross-reactivity to cisplatin and display patterns of antitumor activity and toxicity that differ from those effected by antitumor platinum complexes.

Poster abstract

Organ toxicity of the early-transition metal antitumor agents vanadocene dichloride and titanocene tetrachloroferrate

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Titanocene dichloride is the first representative of organometallic antitumor metallocene complexes that has been investigated preclinically during the past 15 years and was introduced into early clinical studies as cytostatic drug at the end of 1991. It showed antitumor activity against xenografted human gastrointestinal lung and breast carcinomas, failed to induce mentionable myelotoxicity and severe nephrotoxicity in animal studies, but unfolded dose-limiting hepatoxicity.

In the present study we analyzed the preclinical pattern of toxicity of two 'second generation' antitumor metallocene complexes, represented by the neutral complex vanadocene dichloride $[(C_5H_5)_2VCl_2]$ and the ionic titanocene derivative $[(C_5H_5)_2TiCl(NCCH_3)]^+[FeCl_4]^-$. We performed these investigations in mice following intraperitoneal single dose application of the compounds. Whereas vanadocene dichloride induced symptoms of a dose-dependent, functionally relevant nephrotoxicity, the dose-limiting toxicity of the ionic titanocene complex was obviously hepatoxicity which manifested both functionally and structurally. Both complexes induced reversible hypoglycemia, but lacked mentionable myelotoxicity and teratogenicity. There was no remarkable myelosuppression observed after application of both compounds.

These results reveal interesting toxicological properties of both antitumor metallocene complexes, that clearly differ from those of common organic and inorganic platinum antitumor compounds.

A Cyclopropyl-Cobalt(III) to Prop-2-enyl-Cobalt(III) Rearrangement.

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Intramolecular alkylcobalt(III) compounds have general interest as model compounds for coenzyme B_{12}. In coenzyme B_{12} dependent enzyme catalyzed reactions involving carbon skeleton rearrangements a cyclopropyl intermediate has been proposed. Although it is not generally believed that this intermediate is coordinated to the cobalt(III) ion, cyclopropylcobalt(III) compounds have been prepared as model compounds. The cation **A** contains a pendant coordinating cyclopropyl arm which on heating in benzene solution undergoes a ring opening to give a coordinating prop-2-enyl group in the cation **B** as shown in the scheme (py = pyridine).

A   B
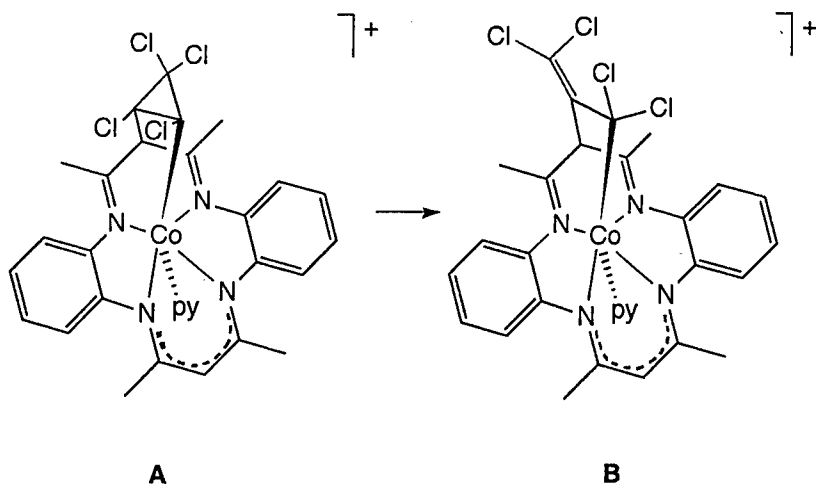
The structure of both the starting material (**A**) and the product (**B**) were established by X-ray crystallography using low temperature diffraction data. The two structure determinations showed that the rearrangement of **A** is analogous to the more common cyclopropylmethyl to but-3-enyl rearrangement.

The Interaction of Mercury(II) with the DNA Dodecamer d(GCCGATATCGGC)$_2$ Studied by ^{1}H NMR Spectroscopy.

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It has been found that both Mn^{2+} and Zn^{2+} appear to interact virtually selectively with G4 in the palindrome dodecamer d(CGCGAATTCGCG) (1,2). However, Hg^{2+} ions interacts with the same sequence in a completely different manner (3). Four Hg^{2+}-ions/per DNA duplex seem to interfere with the Watson-Crick hydrogen bonds of the four central A·T base-pairs of the B-DNA helix. This observation indicate a transition from the normal B-form of the duplex to an apparently new well-defined form interacting with Hg^{2+}.

In order to further probe the sequence-selectivity of Hg(II)- ions the double helix dodecamer d(GCCGATATCGGC) ,with a different AT-context was titrated with aliquots of Hg(ClO$_4$)$_2$ solution. The titration of the oligomer was monitored by ^{1}H-NMR spectroscopy. The ^{1}H-resonance assignments of the original and the Hg^{2+}-form of the dodecamer were carried out by means of two-dimensional COSY/NOESY NMR-techniques.

In the imino region of the ^{1}H spectrum, the T8-NH signal is gradually reduced with a concomitant buildup of a new imino resonance. The T6-NH and G4-NH are also affected, although not as pronounced as the T8-NH. The methyl resonances show only small metal ion induced chemical shifts. However, large chemical shifts are observed for the aromatic proton signals T8-H6 and G4-H8. Significant shifts are also observed for some of the other aromatic and anomeric protons.

The aromatic/sugar region in the NOESY spectra of the Hg^{2+} titrated dodecamer, confirms that T8 and G4 are the most affected bases. This NOESY region also shows that there exist two forms of the duplex at a Hg^{2+}/DNA ratio of 1.6:1.

References:
1: Frøystein, N. Å. and Sletten, E. (1991) *Acta Chem. Scand.* **45**, 219-225.
2: Frøystein, N. Å.,Davis. J. T., Reid, B. R. and Sletten, E. (1993) *Acta Chem. Scand.* **47**, 649-657.
3: Frøystein, N. Å. and Sletten, E. (1994) *J. Am. Chem. Soc.* (in press).

Structures and Reactivity of Dinuclear Copper Coordination Compounds with Poly-imidazole Ligands

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In the course of our research study on copper coordination compounds, we attempt to mimick both the structures and the properties of typeIII dinuclear copper proteins, by modelling the active site of these proteins with imidazole containing ligands. Several new poly-imidazole ligands have been designed and synthesized. With these ligands, various copper coordination compounds (mono-, di-, and hexanuclear) have been obtained [1].

Some very interesting copper complexes have been synthesized with the dinucleating ligand bihm. Crystal-structures of four dinuclear Cu-bihm complexes with different anions have been determined.
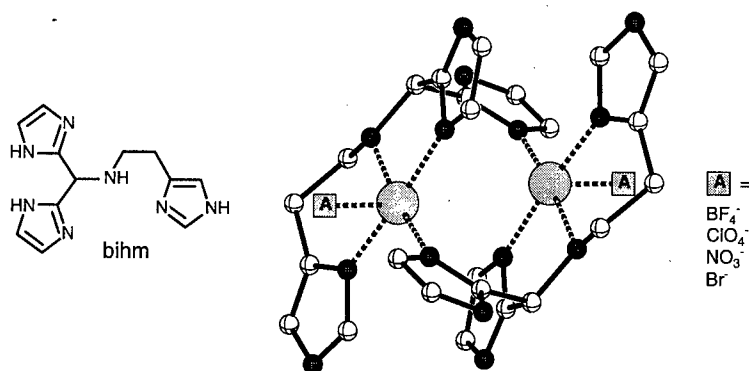
In each dinuclear complex the copper-ion is coordinated by three imidazole nitrogens and one amine nitrogen donor as equatorial ligands and one anion as a weak axial ligand. The Cu-Cu distance is related to the binding strength of the respective anions. It ranges from 3.35 Å for the tetrafluoroborate ion to 3.63 Å for the bromide ion.

The reactivity of the Cu(II) complexes towards hydrogenperoxide and azide as well as the reactivity of the Cu(I) complexes towards dioxygen will be discussed.

[1]   G.J.A.A. Koolhaas, W.L. Driessen, P.J. van Koningsbruggen, J. Reedijk and A.L. Spek.
        J. Chem. Soc. Dalton Trans, **1993**, 3803.

INTERACTION OF COPPER(II) IONS WITH N-PARA NITRO AND N-PARA AMINO- PHENYLSULFONYL DERIVATIVES OF AMINO ACIDS AND PEPTIDES.

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All the effective antibacterial sulfonamides are derivatives of sulfanilamide. Although the usefulness of these compounds was considerably limited by introduction of more efficient antibiotics, recent developments in the application of their metal complexes in burn therapy [1] has revived interest in sulfanilamides and their metal complexes [2,3]. In order to establish the possible interactions of sulfonamide nitrogen with cupric ions, the pH-metric and spectroscopic study was performed on the binding ability of PAS-Gly, PAS-Ala, PAS-Val, PAS-Glu, PAS-Ala-Ala, PAS-Ala-His and PNS-Ala, PNS-Ala-Ala, PNS-Ala-His with Cu(II) ions. In PAS- and PNS-amino acids systems, the $CuLH_{-1}$ and CuL_2H_{-2} species are formed. Cu(II) ions are coordinated to sulfonamide nitrogen below pH 5 forming stable mono- and bis-complexes. The coordination of sulfonamide nitrogen is confirmed by charge transfer band at 380 nm in UV-VIS and CD spectroscopy. The five super hyperfine lines derived from two metal bound nitrogens are observed for pH range in which CuL_2H_{-2} complex predominates. In PAS- and PNS-Ala-Ala systems exist two nitrogen coordination: sulfonamide and peptide nitrogens to Cu(II) ions. Cu(II) ions can deprotonate and bind to a sulfonamide nitrogen from around pH 5. The basicity of sulfonamide nitrogen is lower than that of the peptide amide nitrogen and no distinct anchoring site is necessery to promote the sulfonamide nitrogen deprotonation. In peptide derivatives, the presence of sulfonamide nitrogen and its coordination to the Cu(II) ions can promote the deprotonation and coordination the peptide nitrogen to Cu(II) ions.

1. A.Bult, in *Metal Ions in Biological Systems,* H.Sigel, ed.M.Dekker, New York, vol **16**, 261, (1982).
2. L.Menabue and M.Saladini, *J.Inorg.Biochem.,* **49**, 201, (1993) and refs. therein.
3. T.Kowalik-Jamkowska, H.Kozlowski, L.D.Pettit, K.Pawelczak, M.Makowski, *J.Inorg.Biochem.* in press

COBALT AND IRON COMPLEXES WITH MACROCYCLIC AND CHELATING LIGANDS AS THE BASE OF NOVEL BIOLOGICAL ACTIVE COMPOUNDS. NEW PHYTOIMMUNITY INDUCING AGENTS.

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It is known that active oxygen species (AO) play a key role in some fundamental biological processes such as mutagenesis and some immune reactions. On the other hand, AO is responsible for the increase of plant resistance to fungi pathogens. Recently it has been demonstrated that AO, first of all superoxide, OH-radical and H_2O_2 can contribute to increasing phytoimmunity. E.g., it was shown that infection causes the intensification of AO formation up to fungitoxic concentration on leaf surface. The treatment of plants by some fungicides also increases the generation of AO by leaves, which results in suppression of the disease.

Since transition metal complexes are catalysts of the most important reactions resulting in AO formation, we have suggested that they can be used as starting materials or structural blocks for the creation of new fungicides.

Phytoimmunity inducing properties of new cobalt and iron complexes of phthalocyanine and naphtalocyanine types, were studied in the case of rice blast disease. They included the direct toxic effect of metal complexes on the spores germination of a phytopathogene fungus, viz Pyricularia oryzae; protective action on the isolated rice leaves against infection on addition of metal complexes to inoculum – infection drop with pathogen spores – on the leaf surface; the role of AO in possible mechanism of protection by metal complexes (with this goal AO-scavengers such as superoxide dismutase (for O_2), catalase (for H_2O_2), mannitol, sodium formate (for OH-radical), diazabicyclooctane and methionine, hystidine (for singlet oxygen) was used); effect of the light on the disease-controlling action of complexes.

These results revealed that both direct and plant-mediated generation of AO is the cause by disease-controlling action of metal phthalocyanines.

CYTOCHROME c NITRITE REDUCTASE AND N_2O REDUCTASE: STRUCTURE AND FUNCTION OF THE METAL CENTERS

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Under anoxic conditions, nitrate reduction by prokaryotes proceeds via **Denitrification (1)**, or **Nitrate Ammonification (2)**: (1) $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$; (2) $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$. In *Pseudomonas stutzeri*, the N,N triple bond is formed successively by nitric oxide reductase (NOR) which forms N_2O from NO (the latter being the product of nitrite reductase, NIR) and by nitrous oxide reductase (N2OR) which releases dinitrogen [1]. In *Sulfurospirillum deleyianum*, nitrate is reduced to ammonia, with nitrite as only intermediate. The cytochrome *c* nitrite reductase (Fe-NIR) converts nitrite to ammonia [2]. N2OR is a Cu-containing, dimeric enzyme. 2 Cu atoms/subunit form a mixed-valence chromophore [Cu(1.5)...Cu(1.5)], the so-called Cu_A [3]. Here we report on new structural aspects of Fe-NIR and N2OR, and the reactivity of these enzymes as studied by stopped-flow spectrophotometry.

1. H. Cuypers and W.G. Zumft in: Pseudomonas, Molecular Biology and Biotechnology (Galli, E., Silver, S. and Witholt, B. eds) pp. 188 - 197, American Society for Microbiology, Washington, DC (1992)
2. W. Schumacher, P.M.H. Kroneck Arch.Microbiol., 157, 464 (1992)
3. W.E. Antholine, D.H.W. Kastrau, G.C.M. Steffens, G. Buse, W.G. Zumft and P.M.H. Kroneck Eur.J.Biochem. 209, 875 (1992).

Metal Ions in the Active Site of Inorganic Pyrophosphatase from *Saccharomyces cerevisiae* (as derived from the X-ray study)

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Inorganic pyrophosphatase catalyses the cleavage of high energy pyrophosphate bond in the molecule of inorganic pyrophosphate (PP_i) and thereby shifts towards completion the overall equilibrium of biosynthetic reactions in the cell. The enzyme is active in the presence of bivalent cations only. In the series of bivalent cations the activity decreases as follows: $Mg^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Cd^{2+}$. Ions of Ca^{2+}, UO_2^{2+}, Tb^{3+} and $CaPP_i$ are inhibitors.

The crystal structure of the complexes of inorganic pyrophosphatase from *Saccharomyces cerevisiae* with inhibitors (UO_2^{2+}, Tb^{3+}, $CaPP_i$) and with MnP_i have been studied at 3 Å and 2,4 Å correspondingly. The structure of active site and the centres of binding of metal ions have been described. Based on the positions of three Mn^{2+} ions and phosphate in the active site area the possible role of metal ions in catalysis and plausible mechanism of the enzyme action are discussed.

EFFECT OF POLAR GROUPS ON THE FREQUENCY OF CARBON MONOXIDE COORDINATED BY HEME PROTEINS: THEORETICAL APPROACH.

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It was shown experimentally that the presence of charged or polar groups in heme pocket strongly affects the stretching vibrational frequency of coordinated CO, ν, [1]. To study this effect theoretically, the influence of the point charges, Q, on the electronic structure of the iron-porphyrin complex with imidazole and CO, was studied using the SCF-MO-INDO/S quantum chemical technique [2]. $Q = \pm 0.5$ e⁻, ± 1.0 e⁻ were located in 2.5 - 3.0 Å from the CO oxygen atom, O, in the C-O and perpendicular direction. It was obtained that on the formation of the Fe-CO chemical bond a degree of the orbital charge transfers, OCT, $5\sigma(CO) \rightarrow d_{z^2}(Fe)$ and $d_{\pi}(Fe) \rightarrow 2\pi^*(CO)$ strongly depends on the magnitude and the position of Q. Application of the vibronic theory of activation [3] showed that presence of Q strongly affects ν. For example, location of $Q = -0.5$ e⁻ in 2.5 Å from O increases ν by 25 cm⁻¹, -1.0 e⁻ by 50 cm⁻¹, $Q = +0.5$ e⁻ decreases ν by 30 cm⁻¹ and +1.0 e⁻ - by 65 cm⁻¹. The O - Q distance is another important parameter that controls ν: its increase from 2.5 to 3.0 Å for $Q = +1.0$ e⁻ increases ν by 18 cm⁻¹. At the same time, ν weekly depends on the C-O-Q angle ($\Delta\nu \leq 5$ cm⁻¹).

Reference.

1. G. B. Ray, X.-Y. Li, J. A. Ibers, J. L. Sessler, and T. G. Spiro., J. Am. Chem. Soc., 116 (1994) 162.

2. W. D. Edwards, B. Weiner, and M. C. Zerner., J. Am. Chem. Soc., 108 (1986) 2196.

3. S.S. Stavrov, I.P. Decusar, and I.B. Bersuker. New J. Chem., 17 (1993) 71.

HEAVY METALS CONTENTS IN THE FEMUR CAPITULUM OF PEOPLE LIVING INDUSTRIAL REGION.

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Environmental factors can exert a specific influence on bone metabolic and cumulation Pb, Cd, Ni, Zn, Cu, Cr, Mn, Fe. Analysis of geometric mean of these element contents coefficient of varibility and range of variations made possible to divide these metals into two groups: those with greater (Zn, Cr, Fe, Pb) and those with lower level of contents (Cd, Cu, Co, Mn).

The range of the change of concentration given metals were following:

Pb: 61,39 - 122,72 μg/g   Mn: 9,2 - 74,6 μg/g

Cd: 3,55 - 32,12 μg/g   Cr: 50,89 - 482,7 μg/g

Ni: 8,96 - 139,05 μg/g   Fe: 76,17 - 583,9 μg/g

Cu: 6,76 - 40,13 μg/g   Co: 10,4 - 138,3 μg/g

Zn: 184,4 - 1145 μg/g

Lead contents in relation occurrence in air may be expressed by the equation:

$$C_b = E \cdot K \cdot M (C_a + 1.65)^T$$

where:

E - coefficient of ecotoxicological estimation:

K - coefficient of Pb contamination in the system bone-blood:

M - estimation coefficient for the system blood-air: for Pb = 16.1

C_a - Pb content in the air

T - coefficient expressing Pb content in the blood as a function of Pb content in the air; for maximum Pb occurrence levels in the blood, $T = 0.740$, for minimum levels $T = 0.206$.

Heavy metals in human blood in secondary emission of dust conditions

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Hazards for man and other living organism may originate from prolonged low-level exposures to heavy metals relased into the environment as a result of various industrial processes and increassing traffic density.

In Upper Silesia the most important anthropogenic sources of heavy metals are the air emission and secondary emmision of deposited dust.

The recent studies have indicated that in long-term, low-dose exposure heavy metals may act as a cumulative toxic efects. Dust containing Pb, Cd, Zn, Ni, Cr, Mn, deposited on streets surface, can to be the cause secondary strong in crease of concentration of thees elements in low layer of air.

The range of change of speed secondary emission of heavy metals contained in deposited dust is dependent from weather (wind, rain) and quantity of deposited dust.

The hypothesis of common source for some heavy metals in dust deposited, suspended dust and in blood confirmed by high correlation between thees metals at the investigation samples. Because blood is a suitable indicator medium to monitor human heavy metals exposure.

The highest metals blood levels were found in subjects living very close to the polluted streets and smalting plants.

The Redox Reactivity of the Type 1 (blue) Copper Protein Amicyanin from *Thiobacillus versutus* with Small Inorganic Complexes by Kinetic and ^{1}H NMR Studies.

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Amicyanin is a type 1 (blue) Cu protein which mediates electron transport between methylamine dehydrogenase (MADH) and c-type cytochromes of certain methylotrophic bacteria. In the redox cycle the cofactor TTQ of MADH oxidises methylamine according to Eqn (1):

$$CH_3NH_3^+ + H_2O \rightarrow NH_4^+ + CH_2O + 2H^+ + 2e^- \qquad (1)$$
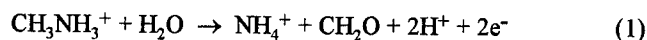
Amicyanin from *Thiobacillus versutus* has a molecular weight of ~11.7 kDa, typical spectroscopic properties of a type 1 Cu site, and a reduction potential of 260 mV at pH 7.0. The Cu atom is coordinated by His-54, Cys-93, His-96 and Met-99.

In this work the redox reactivity of reduced amicyanin, AmCu(I), with $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$ has been studied by stopped-flow spectrophotometry. A loss in reactivity is observed at low pH values due to protonation and dissociation of the Cu-coordinating His-96 (pK_a 6.6) in reasonable agreement with a value of 6.9 from ^{1}H NMR studies.[1] Corresponding active site effects have been reported in the case of plastocyanin (pK_a 4.9)[2] and more recently of pseudoazurin (pK_a 4.7).[3] However, no corresponding acid dissociation has been detected for five other type 1 Cu proteins in the accessible range of pH. A possible correlation with the spacing between residues coordinating the Cu is noted. The higher pK_a in amicyanin may be linked with the need to transfer electrons and protons from the TTQ cofactor of MADH at pH ~7.0.

Although the amino-acid charge balance in AmCu(I) is -4 there are a number of basic residues in the locality of the hydrophobic His-96 and Phe-92. Line-broadening of certain peaks in the ^{1}H NMR spectrum of AmCu(I) in the presence of redox inactive $[Cr(CN)_6]^{3-}$ highlights the importance of these residues for reaction with $[Fe(CN)_6]^{3-}$. From the structure there are four basic residues (1 Arg and 3 Lys) close to the exposed Phe-92. Saturation kinetics are observed for the $[Fe(CN)_6]^{3-}$ oxidation of AmCu(I) at pH 5.2. Further support for an involvement of this site comes from competitive inhibition studies with $[Cr(CN)_6]^{3-}$. The possibility of a dual-site reactivity (at His-96 and Phe-92) will be discussed.

1. A. Lommen and G.W. Canters, *J. Biol. Chem.*, 1990, 265, 2768.
2. A.G. Sykes, *Struct. Bonding (Berlin)*, 1991, 75, 175.
3. C. Dennison, T. Kohzuma, W. McFarlane, S. Suzuki and A.G. Sykes, *J. Chem. Soc., Chem. Commun.*, 1994, 581.

^{1}H NMR of the ferredoxin from the hyperthermophilic archaeon *Pyrococcus furiosus*: Electronic structure and participation of the disulfide bridge in the redox cycle.

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The 66 amino acid residue ferredoxin, Fd, from the hyperthermophilic archaeon *Pyrococcus furiosus*, *Pf*, has been investigated by 1D and 2D NMR methods. The organism thrives optimally above 100°C in sulfide-rich marine environment, and the isolated Fd is stable to any denaturation over 24 h at 95°C. The sequence shows that only three of the four Cys in the consensus sequence for the four-iron cluster are present, with one position occupied by Asp (position 14). Two additional Cys are thought to participate in a disulfide bridge. The presence of only three ligated Cys for the cluster allows facile interconversion between 3Fe and 4Fe forms (1). 2D NMR methods have allowed the location of signals for all but two residues in the protein, and standard sequence-specific assignments identify 56 of these residues. Backbone NOEs reveal a secondary structure which is similar to that of the *Desulfovibrio gigas* Fd, but with extension of numerous secondary structural motifs; the presence of a disulfide link involving Cys 21 and Cys 48 could be confirmed (2). The assignment of the cluster ligated Cys signals shows that the sequence basis of the magnetic asymmetry in the 3Fe oxidized Fd is not the same as that from hyperthermophilic archeon, *Thermococcus litoralis* (3). The 4Fe *Pf* Fd undergoes four discrete redox steps for which the cluster is oxidized, $[Fe_4S_4]^{+2}$, in two, and reduced, $[Fe_4S_4]^{+1}$, in the other two states. Thiol titration reveals that the alternate redox states for a given cluster oxidation state differ in having zero and two free cysteines. Hence, both the cluster and disulfide bridge are redox active in *Pf* 4Fe Fd. At ambient temperatures, the population of the four alternate disulfide redox states are kinetically controlled, and it is possible to isolate and retain for extended periods of time each of the four redox states in nearly pure form for spectroscopic and physico-chemical investigations.

(1)   Conover, Kowal, Fu, Park, Aono, Adams & Johnson, (1990) J. Biol. Chem. **265**, 8533.

(2)   Teng, Zhou, Smith, Busse, Howard, Adams, & La Mar (1994) Biochemistry **33**, in press.

(3)   Donaire, Gorst, Zhou, Adams, & La Mar (1994) J. Am. Chem. Soc. **116**, in press.

Structural Features of Two Haem Proteins

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Proteins containing haem groups are known for their diversity on biological function that is intimately associated with the haem group and its environment. We will report the results from recent structure determinations of two haem proteins by X-ray diffraction methods.

Coprinus cinereus peroxidase (CiP) is the only peroxidase isolated from the ink cap basidiomycete, *Coprinus cinereus*. It consists of a single chain of 343 amino acids, one haem group and two Ca^{2+} ions that are important for protein folding. The crystal structure has been determined for a recombinant form of *Coprinus cinereus* peroxidase expressed in *Aspergillus oryzae* to a nominal resolution of 2.0 Å. Two crystallographically independent molecules related by pseudotranslational symmetry are found in the crystals, which provides two independent determinations of the haem environment. In its resting state the haem group contains Fe(III) with His183 as an axial ligand. Like the other peroxidase structures a water molecule is found in the position corresponding to a possible sixth ligand. The haem environment in CiP will be compared to other known peroxidase structures and related to their enzymatic differences.

Cytochrome c_4 from *Pseudomonas stutzeri* is known to play a role in the electron transport processes. It consists of one amino acid chain of 190 amino acid residues with two haem groups covalently bound. It is the first structure of a cytochrome c_4 determined to a higher resolution (2.2 Å). The crystals contain two independent molecules. The two haem groups within a molecule have similar coordination with His and Met as the axial ligands. The distance between the two Fe centers is 19 Å, the angle between the planes of the porphyrine rings is approx. 31°. Within the molecule the haem groups are interacting through a short hydrogen bond between the propionate groups. The crystal packing gives rise to interesting interactions between haem groups from different molecules. Two of the haem groups are almost parallel with a short Fe-Fe distance of 16 Å.

KINETIC AND SPECTROSCOPIC STUDIES OF BACTERIOFERRITIN AND ITS SITE-DIRECTED VARIANTS

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Bacterioferritin (BFR) is an iron storage protein found in a wide variety of bacteria. The protein consists of 24 identical subunits arranged to form a highly symmetrical, approximately spherical molecule surrounding a central cavity.

Previous spectroscopic studies of BFR have indicated that each BFR subunit contains a binuclear iron centre, which is important in iron uptake and oxidation by BFR (Le Brun *et al.*, 1993a; 1993b). Molecular modelling studies have identified a plausible site for the binuclear centre, which is located within the four α-helical bundle of each subunit (Cheesman *et al.*, 1993) and has similarities to the binuclear iron centres in the R2 subunit of ribonucleotide reductase and the hydroxylase subunit of methane monooxygenase.

Studies are presented of site-directed variants of BFR in which residues proposed to be involved in iron ligation in the binuclear site have been replaced by non complexing residues. Results confirm the proposed location of the iron centre, and advance our understanding of the iron uptake process in BFR.

References
1. Le Brun, N. E., Cheesman, M. R., Thomson, A. J., Moore, G. R., Andrews, S. C., Guest, J. R. and Harrison, P. M. *FEBS Lett* **323**, 261-266 (1993a).
2. Le Brun, N. E., Wilson, M. T., Andrews, S. C., Guest, J. R., Harrison, P. M., Thomson, A. J. and Moore, G. R. *FEBS Lett.* **333**, 197-202 (1993b).
3. Cheesman, M. R., Le Brun, N. E., Kadir, F. H. A., Thomson, A. J., Moore. G. R., Andrews, S. C., Guest, J. R., Harrison, P. M., Smith, J. M. A. and Yewdall, S. J. *Biochem. J.* **292**, 47-56 (1993).

ANTISENSE OLIGONUCLEOTIDES LINKED TO METAL CHELATES AS POTENTIAL DRUGS FOR CHEMOTHERAPY OF CANCER AND VIRAL DISEASES

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Gene expression is controlled in-vivo by regulatory proteins that possess the properties of recognition and binding to specific sequences in gene. One can expect to interfere with the expression of a selected gene if one can design a molecule that can recognize and compete at the binding of regulatory proteins to the target gene. Synthetic antisense or antigene oligonucleotides constructed with appropriate sequences are molecules that are capable to recognize and bind to complementary sequences in target DNA or RNA. Recognition of duplex structures can be achieved by triple helices forming oligonucleotides (TFO) provided the target region in DNA contains stretches of contiguous purines or pyrimidines. The efficiency of the antisense or the antigene effects can be greatly increased if, besides binding to target sequences, the oligonucleotide molecules can create irreversible damages on the target nucleic acids. Along these lines, reactive oligonucleotides were developed by covalent linkage of chemically or photochemically activatable groups at the 5' or the 3' end of oligonucleotides. The sequence recognition is ensured by the oligonucleotide part while the metal moiety acts as a DNA damaging center.

Chemically activatable compounds are represented mainly by metal chelates such as : Iron-Ethylendiaminetetraacetic acid (Fe-EDTA), Copper-Phenanthroline (Cu-PHEN), Porphyrins (Fe or Mn-POR) and some other metal chelates. These metal complexes have been covalently linked to oligonucleotides. In in-vitro assays it has been shown that target DNA hybridized to oligonucleotides-metal-chelates were degraded in the presence of reducing (or oxidizing) agents in aerated solutions. The reaction was found to be sequence-specific as cleavage of the target nucleic acid was only observed at the binding site of the oligonucleotide-metal chelate conjugates.

Photochemical activation is possible with Porphyrin-oligonucleotide conjugates when the porphyrin moiety is under its free base form or metallated with Zn(II) or Pd(II). The photochemical damages consisted in mainly crosslinks species and bases oxidation. The reaction was found to be specific as only G bases that are in the vicinity of the porphyrin molecule are modified.

Viruses and especially the HIV are among the most interesting targets for reactive oligonucleotides. This is because that viral sequences are not likely to be found in host genome and TFO (Triple-helix forming oligonucleotide) are unique molecules that are capable to recognize integrated viral sequences, a total of 9200 base pairs among the 3 billions bp of human genome.

In 1992 the first administration of antisenses in man has been achieved in a case of severe form of leukemia (AML). A clinical phase I/II trial is underway in France and in the US for assessing the toxicology of an anti-HIV antisense. For the last 2 years many studies on the efficacy of antisenses in curing animals with implanted tumors have appeared. These advanced studies showed up several issues that should be overcome for future clinical applications of the antisense strategy. Among solutions that should be brought to progress in this field, antisenses equipped with metal chelates could play a positive role in recognition and destruction of deleterious viral genes in seropositive individuals.

$[Fe^{II}(TIM)(OAC)]ClO_4$ AND $[Fe^{II}(TIM)\,(PHENOAC)]ClO_4$: THE FIRST FE(II) COMPOUNDS MODELLING THE BIDENTATE CARBOXYLATE AND FOUR IMIDAZOLE DONOR SET OF THE PHOTOSYNTHETIC MONONUCLEAR NON-HEME FERROUS SITES

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The reactions centers of photosynthetic bacteria and the photosystem 2 of oxygenic photosynthetic organisms include a ferrous ion between the primary and the secondary quinone electron acceptors. The X-ray molecular structure determination of the reaction center of Rhodopseudomonas Viridis[1] and R. Sphaeroides[2] indicates that the ferrous ion is in a distorted octahedral ligand environment including four nitrogen atoms pertaining to the imidazole moiety of histidine residues and two oxygen atoms from a glutamic acid residue of the surrounding protein.

Owing to the interest of ferrous complexes as models of the ferrous ion of mononuclear non-heme iron containing proteins, we have synthesized and studied several chelates resulting from the reaction of bis[(imidazol-4'-methyl)-4 imidazol-2-yl] methane (TIM)[3] with ferrous salts. In this communication, we report the synthesis and Mössbauer and variable temperature magnetic susceptibility results for $[Fe^{II}(TIM)(OAc)]ClO_4$ (1), $[Fe^{II}(TIM)(phenOAc)]ClO_4$ (2), $Fe^{II}(TIM)(OAc)_2$ (3) $Fe^{II}(TIM)(HCO_2)_2$ (4), $Fe^{II}_2(TIM)_2(C_2O_4)$ (5). X-ray crystal structure determination of 1 and 2 has been performed and a detailed comparison with the ferrous site of the reaction center of Rhodopseudomonas Viridis has been carried out. The variation in the Mössbauer properties among this set of high-spin iron(II) complexes compared to those of photosynthetic bacterial reaction centers and PS2 particles indicates that in addition to the nature of the ligands the distortion of the Fe(II) coordination sphere is of paramount importance in designing proper structural and spectroscopic analogues of the photosynthetic mononuclear non-heme ferrous sites.

1) H. Michel, O. Epp, J. Deisenhofer, *EMBO J.,* **5**, 2445 (1986). 2) J. P. Allen, G. Feher, T. O. Yeates, H. Komiya, D. C. Rees, *Proc. Natl. Sci. USA.,* **85**, 8487 (1988). 3) E. Mulliez, *Tetrahedron Letters,* **30**, 6169 (1989).

MECHANISM OF THE B_{12}-DEPENDENT POISONING ENVIRONMENT BY ALKYL DERIVATIVES OF HEAVY METALS.

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It is well known that pollution of the environment with toxic methyl derivatives of mercury and other heavy metals is due to the contact of B_{12}-synthesising bacteria with industrial waste. Wood *et al.* showed (see *e.g.* [1]) that the process in question is a stoichiometric transalkylation reaction between methylcobalamin and corresponding metal ions. Later Fanchiang [1] suggested, on the ground of kinetic data, that it proceeds *via* formation of charge-transfer complex intermediate involving a highly oxidized form of methylcobalamin. Although such labile oxidized forms are known for organocobalt complexes derived from models of B_{12}, numerous attempts to establish their existence in its own series failed [1, 2], probably due to experimental difficulties.

We have recently succeeded in filling that gap. Namely, we observed formation of extra labile species during electrochemical or low temperature chemical oxidation of hydrophobic analogs of alkylcobalamins, *viz.* Co-alkyl derivatives of heptamethyl ester of cobyric acid. Their nature as products of one-electron oxidation of the latter compounds was proved by cyclic voltammetry. Analysis of their ESR spectra in frozen solutions revealed their electronic structure as organocobalt(IV) complexes. Thus, the mechanism of formation of toxic organic derivatives of heavy metals in the environment is now properly established.

1. Y.-T.Fanchiang, *Coord. Chem. Rev.*, **68**, 131 (1985), and references therein. 2. M.E.Vol'pin *et al.*, *J. Organometal. Chem.*, **289**, 263 (1985), and references therein.

Diastereomeric DNA Binding Geometries of Tris(1,10-phenanthroline) Ruthenium(II) and Related Complexes

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The nature of the binding of the propeller-shaped tris(1,10-phenanthroline) Ru(II) enantiomers to DNA continues to be a controversial question; almost all conceivable DNA binding modes have been suggested for the one or the other enantiomer over the years. The Ru(phen)3 chromophore has intense electronic transitions polarised in all three dimensions, producing linear dichroism spectra for the flow-oriented DNA complexes that are very sensitive to the binding geometry. However, the presence of degenerate, or near degenerate and strongly overlapping transitions and electronic perturbation effects precludes a quantitative evaluation of LD in terms of binding geometry. To overcome this problem we have used photoselection anisotropy as a complement to linear dichroism to estimate the overlap and confirm the assignment of different transitions in the DNA-perturbed Ru(phen)3 chromophore.

USE OF CD SPECTROSCOPY TO STUDY THE TRANSITION METAL CENTERS OF THE MITOCHONDRIAL RESPIRATORY CHAIN

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All bc_1 complexes contain four redox centers: the 'Rieske' [2Fe-2S] cluster, cytochrome c_1, and two heme b centers. In whole bc_1 complex, the 'Rieske' [2Fe-2S] cluster cannot be observed in the UV/Vis absorption spectrum since this is dominated by the intense heme $\pi \rightarrow \pi^*$ transitions. Therefore, EPR spectroscopy is generally used to study the 'Rieske' cluster in the bc_1 complex. This method has the disadvantage that it is confined to the reduced state of the cluster while the oxidised state is EPR silent.

Both the oxidised and the reduced 'Rieske' [2Fe-2S] cluster have a high natural CD intensity. We have therefore used CD spectroscopy to investigate the 'Rieske' cluster within the bc_1 complex. The cluster has two groups with redox-dependent pK values of 7.7 and 9.1 on the oxidised protein and pK > 10 on the reduced protein[1]. The protonation state can be monitored by CD spectroscopy[2]. The pK values must belong to residues in close contact with the cluster, most likely to the two histidine ligands of one iron atom. Upon reduction at neutral pH, the [2Fe-2S] cluster will take up one proton which is released upon re-oxidation.

The [2Fe-2S] cluster and heme b_L constitute a common reaction site ('Q_o center') where oxidation of hydroquinone (UQH_2) and bifurcation of the electron path takes place. Since this oxidation requires deprotonation of UQH_2, the redox-dependent pK values are considered to be essential for the electron transfer mechanism.

(1) T A Link, W R Hagen, A J Pierik, C Assmann, G von Jagow, Eur. J. Biochem. 1992, 208, 685.

(2) T A Link, Biochim. Biophys. Acta 1994, 1185, 81.

Purification and characterization of the representative enzymes isolated from a Sulfate Reducing Bacteria present in a biocorroded microcosmos

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Sulfate Reducing Bacteria (SRB) has been described as one of the more frequently implicated microorganisms in microbial corrosion of iron and ferrous alloys (1).

Previous work reported the involvement of a SRB recovered from a corroded ship hull moored off in the Indonesian coast (2). The role of hydrogenase enzyme as well as the possible involvement of bacterial exopolymers in the corrosion process has been discussed (3,4).

So far, very little information is available on the enzymatic equipment of this bacterium.

The understanding of the fundamental processes involved in SRB influenced corrosion justifies the biochemical and structural studies of the representative enzymes involved in the sulfate reduction pathway.

In this work, we repport a preliminary study on the purification of several enzymes isolated from this bacterial strain. Biochemical and spectroscopic characterization of APS reductase, sulfite reductase, hydrogenase, ferredoxin and cytochrome c_3 are under way.

[1]Hamilton, W. A. (1985) *Ann. Rev. Microbiol.* **39**, 195-217
[2]Beech, I.B., S. Cheung, C. P. Chiu, C. C. San, M. A. Hill, R. Franco and A. R. Lino, International Symposium on Marine Biofouling and Corrosion, University of Portshmouth,13-16 July 1993
[3]Cheung, S. and I. Beech, Third European Federation of Corrosion Workshop on Microbial Corrosion , Portugal, 13-16 March 1994.
[4]Franco, R., I. Moura, J.LeGall, H.D. Peck, Jr., B. H. Huynh and J.J.G. Moura, (1993),*Biochim. Biophys. Acta*,**1114**, 302-308.

INTERMEDIATES IN THE COPPER CATALYZED OXYGENATION OF FLAVONOL IN RELATION TO QUERCETINASE ACTION

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Quercetin 2,3-dioxygenase is a copper(II)-containing enzyme which metabolizes quercetin to a depside and carbon monoxide by the use of molecular oxygen. Literature data suggest that in the enzyme reaction an *endo*-peroxide is formed (path a) which leads to the depside by extrusion of carbon monoxide. However, there were also suggestions that the cleavage reaction may have a different route (path b) giving 1,2-dioxetaneand ketocarboxylic acid intermediates and finally the depside.[1]
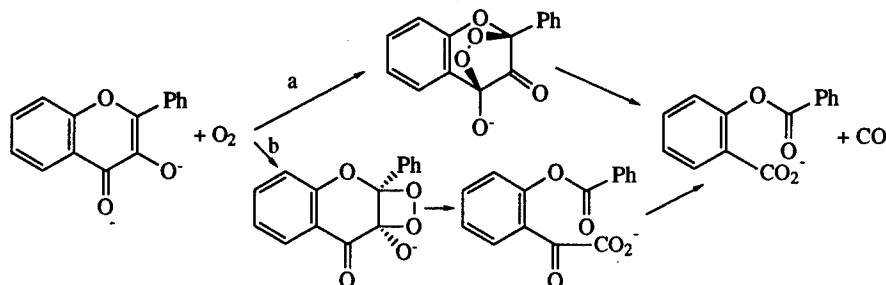
In the course of our studies on model systems we have found that in the reaction of flavonol (flaH) with metallic copper, in the presence of ligands such as triphenylphosphine (PPh_3), 2,2'-bipyridine (bpy), and N,N,N',N'-tetramethyl-ethylenediamine (tmeda), first the compounds $[Cu(fla^{\bullet-})L_2]$ and then $[Cu(fla)L_2]$ are formed. The oxygenation of $[Cu(fla^{\bullet-})(PPh_3)_2]$ and $[Cu(fla)(PPh_3)_2]$ leads to $[Cu(O-bs)(PPh_3)_2]$ (O-bs = O-benzoylsalicylate). In the oxygenation of $[Cu(fla)(bpy)]$ the compound $[Cu(fla)(2-OH-C_6H_4COCO_2)(bpy)]$ (1) was isolated and the structure determined by x-ray diffraction. When $[Cu(fla)(tmeda)]$ was oxygenated the copper carbonyl complex $[Cu(fla)(CO)(tmeda)]$ (2) could be isolated. X-ray structure

(1)
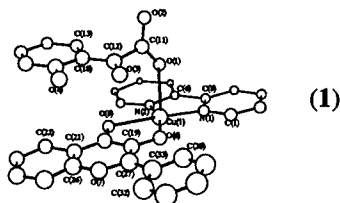
determination is in progress. The compounds isolated and characterized suggest that the reaction path b, as shown in the scheme above, seems to be appropriate in the present system and so far the copper mediated cleavage process is concerned it has to be taken into consideration.

Details of the chemistry summarized here will be discussed in detail at the meeting.

1. É. Balogh-Hergovich and G. Speier, *J. Mol. Catal.* **1992**, *71*, 1-5

Structural and Mechanistic Studies of Methane Monooxygenase

by

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Abstract: The three dimensional X-ray structure of the hydroxylase
component of methane monooxygenase from *Methylococcus capsulatus* (Bath) has
been determined, revealing the geometry of the catalytic diiron center at the
active site, a hydrophobic pocket for binding of substrates, a possible route for
methane access through the α subunit to this hydrophobic cavity, and amino acid
residues that might partake in the catalytic mechanism. Stopped flow optical
spectroscopic and freeze-quench Mössbauer investigations of the reaction of
dioxygen with the reduced, diiron(II) hydroxylase in the absence of substrate
were carried out. Two diamagnetic intermediates, designated L and Q, have
been characterized and are tentatively assigned. The reaction cycle proceeds in
the following manner: $Fe(II)_2 \rightarrow L \rightarrow Q \rightarrow Fe(III)_2$. In the presence of substrate, Q
reacts to give product alcohol. Application of radical clock substrate probes to
study the hydroxylation step indicated that, if radicals form in the reaction
mechanism, they will have exceedingly short lifetimes, $< 10^{-13}$ sec. These and
other results lead to the following working hypothesis for the reaction
mechanism. The resting state of the hydroxylase is reduced by two electrons in
the presence of substrate and other protein components to a diiron(II) center in
which the protein ligands are still coordinated but in which carboxylate shifts
have occurred to lead to two bridging glutamates. Dioxygen coordinates to the
open sites and is reduced to form a bridged diiron(III) peroxide intermediate or
its equivalent (compound L). This species is further activated reductively by a
nearby cysteine residue in the active site with O-O bond cleavage to form a
metallooxyl, formally an analogue of the hydroxyl radical, a seven-electron O^{-}·
species (compound Q, which could also be formulated as a high valent iron oxo
intermediate). The metallooxyl and cysteinyl radical formed in this step then
attack the C-H bond in a concerted fashion, restoring the cysteine residue and
leading to coordinated methoxide, which is protonated and leaves as methanol.
This work was supported by grants from the National Institute of General
Medical Sciences and Shell. References: Rosenzweig, A. C.; Frederick, C. A.;
Lippard, S. J.; Nordlund, P. *Nature*, **1993**, *366*, 537-543; Feig, A. L.; Lippard, S. J.,
Chem. Rev., **1994**, *94*, 759-805.

METAL-MODIFIED NUCLEOBASE PAIRS

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Hydrogen bonding between nucleobases is of paramount importance in many biological processes involving nucleic acids, examples being DNA replication, DNA transcription, RNA translation, and reverse transcription. Apart from the classical Watson-Crick and Hoogsteen base pairing patterns, many additional ones have been discovered lately, which include triples and various forms of quartets, for example.

Insertion of a metal of suitable geometry into a nucleobase pair or displacement of a proton normally involved in H bonding in a base pair yields "metal-modified nucleobase pairs". An essential feature of a base pair - planar or nearly planar orientation of the two bases - is maintained. Frequently one of the original H bonds is still kept. Our interest in "metal-modified base pairs" relates both to their possible relevance in biology and feasible applications.
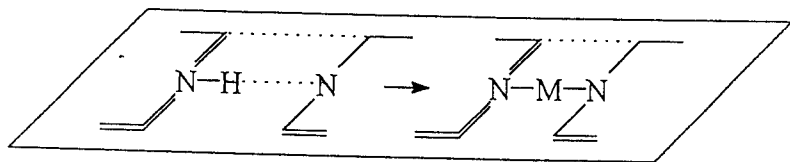
We have recently described examples of metal-modified base pairs containing the linear trans-(amine)2Pt(II) entity [1-4] or a distorted trigonal-planar Ag(I) ion . [5] We have now extended this list of examples to tetrahedral Zn(II) ions. [6] Insertion of a tetrahedral metal ion in a planar base pair is possible only if the nucleobases bound to the metal are coordinated in different fashions, viz. one base via an endocyclic nitrogen and one base via an exocyclic group.

References

(1) Dieter-Wurm, I.; Sabat, M.; Lippert, B. *J. Am. Chem. Soc.* **1992**, 114, 357.
(2) Krizanovic, O.; Sabat, M.; Beyerle-Pfnür, R.; Lippert, B. *J. Am. Chem. Soc.* **1993**, 115, 5538.
(3) Schreiber, A.; Hillgeris, E. C.; Lippert, B. *Z. Naturforsch.* **1993**, 48b, 1603.
(4) Schreiber, A.; Erxleben, A.; Fusch, E. C.; Lippert, B. to be published.
(5) Menzer, S.; Sabat, M.; Lippert, B. *J. Am. Chem. Soc.* **1992**, 114, 4644.
(6) Fusch, E. C.; Lippert, B., submitted for publication.

SMALL SYNTHETIC HEME-PROTEINS BY COVALENT BINDING OF STRUCTURED PEPTIDES TO PORPHYRINS

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Using the recent knowledges on molecular architecture, it is possible to project peptides of medium size with well defined tertiary structure that should be able to coordinate metal atoms reproducing the structural topology in the active site of the biological systems.

We have projected and synthetized a bis-chelated deuteroporphyrin covalently linked with two homologues nonapeptides (see figure).
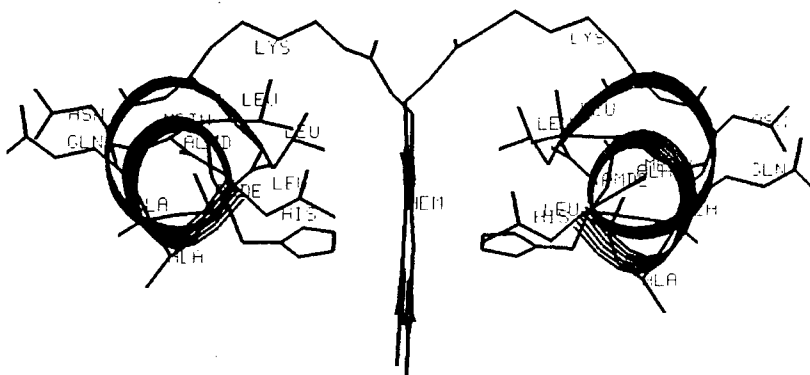
Circular Dichroism measurements are indicative for an α-helicoidal conformation for both the peptidic chains. Moreover Uv-vis spectra and Raman studies are in agreement with a bis-Histidine axial coordination with low spin state both for Fe(III) and for Fe(II), suggesting a conformation of the whole molecule close to that projected.

Some chemical and physical properties like the redox behaviour and the accessibility of the iron in the hydrophobic pocket have been investigated and are compared with that of natural heme-proteins.

STUDIES ON COMPLEX FORMATION IN THE Cu(II)/ POLYAMINE/ADENOSINE TERNARY SYSTEMS

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Aliphatic polyamines (PA): putrescine - $NH_2(CH_2)_4NH_2$ (Put), spermidine - $NH_2(CH_2)_3NH(CH_2)_4NH_2$ (Spd) and spermine - $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ (Spm) are natural components of most living organisms. These compounds play an important role in a number of biochemical processes, particularly in the transfer of genetic information. Centres of PA interaction with nucleic acids are at the same time potential coordination sites of metal ions occurring in living cells.

The stability constants (calculated on the basis of a computer analysis of potentiometric data) of the systems Cu(II)/Put/Ado and Cu(II)/Spd/Ado (Ado = adenosine) and mode of coordination have been determined. Apart from the nitrogen atoms from PA, characteristic coordination sites are N(1) and N(7) atoms from Ado (EPR, 1H NMR, 13C NMR, UV-VIS). At most four nitrogen atoms are involved in coordination. Increasing length of polyamine chain diminishes the tendency to form of mixed complexes. Moreover, the polyamine presence modifies the coordination character of the nucleoside. A polymeric complex $Cu(Ado)_n$, typical of binary systems is not formed in the system Cu(II)/Spd/Ado. Characteristic differences in polyamine interactions are observed. In ternary systems with Spd the coordinative dichotomy (bonding through N(1) or N(7) atoms of the nucleoside) found in Cu(II)/Ado, disappears. The presence of putrescine extends the pH range in which the dichotomy occurs. The non-covalent ligand -ligand interactions were observed in the investigated systems (e.g. the formation of Put-Ado adduct was detected).

A STUDY OF THE INTERACTION OF STREPTONIGRIN AND BIPYRIDINE ANALOGUES WITH ZINC IONS AND DNA

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Streptonigrin **1** is an anti-cancer agent, effective against a broad spectrum of cancers as well as several viruses.[1] Studies *in vivo* and *in vitro* suggest that streptonigrin exerts its cytotoxic effect by interacting with and cleaving DNA. This process requires oxygen, reductively activated streptonigrin and divalent transition metal ions.[1] The mode of action, especially the role of the metal ions, is not understood.

We have determined the solution structure of streptonigrin,[2] and are currently studying the metal complexes of **1** and their interaction(s) with DNA using high field NMR spectroscopy. Preliminary results indicate that streptonigrin forms several complexes with zinc ions, the most stable complex involving the bipyridyl chelate. In order to assist the characterisation of the metal complexes of **1**, the coordination chemistry of ligands **2**[3] and **3**, which contain the central coordination sites of streptonigrin, have been extensively studied. These results, as well as studies of the interaction of DNA subunits and oligonucleotides with Zn^{2+} and streptonigrin, will be presented.

1

2 R=CH$_3$
3 R=COOCH$_3$

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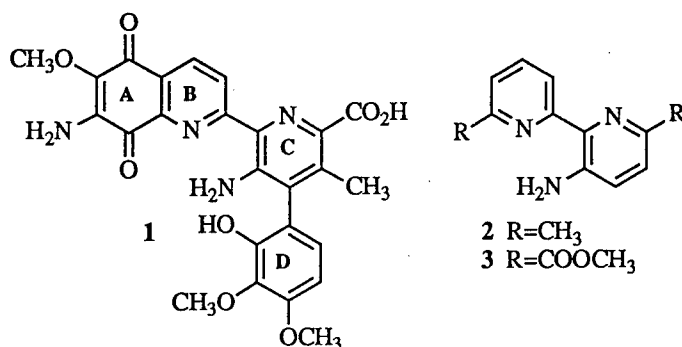
[1] Hajdu, J. *Metal Ions in Biological Systems* **1985**, 53-80 and references cited therein.

[2] Harding, M.M.; Long, G.V.; Brown, C.L. "Solution Conformation of the Antitumor Drug Streptonigrin" *J. Med. Chem.* **1993**, *36*, 3056-3060.

[3] Long, G.V.; Boyd, S.E.; Harding, M.M.; Buys, I.E.;Hambley, T.W. "Synthesis, Properties and Complexation Studies of 3-Amino-6,6'-dimethyl-2,2'-bipyridine" *J. Chem. Soc., Dalton Trans.* **1993**, *21*, 3175-3180.

NUMBER OF REDOX-BHOR PROTONS IN TETRAHEME CYTOCHROME c_3

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Tetraheme cytochrome c_3 is a small protein with approximately 14 kDa, characteristic of the *Desulfovibrio* genera. The hemes have a bis-histidinil axial co-ordination and very low redox potentials. It was shown by X-ray and NMR that the spatial arrangement of the hemes is highly conserved, although the amino-acid sequences have very low homology. [1]

The finding that the redox potentials of the various hemes are pH dependent[2] (redox-Bhor effect), suggest a heterotropic electron-proton coupling function for this protein, enabling a synchronised transfer of electron and protons in energy transduction processes.[3]

Results obtained using potentiometric methods are in good agreement with available NMR data, showing that near physiological pH there are two protons involved in the redox-Bhor effect.

These findings have important consequences in interpreting the regulatory behaviour of cytocromes c_3 and their function in coupling to the enzyme hidrogenase which catalyses the reaction $2H^+ + 2e^- \leftrightharpoons H_2$ and plays a pivotal role in the energy transduction processes of sulphate reducing bacteria.

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[1]Moore, Geoffrey R., Pettigrew, Graham W. (1990) in *Cytocromes c - Evolutionary, structural and physicochemical aspects* pp. 138 -139, Springer-Verlag.

[2]Santos, Helena, *et all*, (1984) *Eur. J. Biochem. 141*, 283-296.

[3]Xavier, António A. (1986) *J. Inorg. Biochem. 28*, 239-243.

BEYOND THE STRUCTURE...

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Formulating hypotheses on how a protein or an enzyme functions is certainly helped by the availability of gross structural information as it can be obtained from an X-ray structure. When a variety of X-ray structures of the same protein system under different conditions are available, a richer picture always emerges. Similar considerations hold for solution structures, as well as for any kind of structural information in solution. However, the individual structures should be better regarded as hints for the mechanism rather than as detailed representations of true intermediates or transition states. Discrepancies and seeming inconsistencies within solution data, solid state data, or between X-ray and solution structures should be considered in this light.

The concept will be developed by showing two striking examples of disagreement between solution and solid state data, which are absolutely informative to support apparently unrelated enzymatic mechanisms. The first example is the structure of anionic inhibitor adducts of carbonic anhydrase, where the inhibitor is not bound to the metal in the solid state and bound to the metal in solution. The second is the structure of reduced copper-zinc superoxide dismutase, where the histidinato bridge between copper and zinc is clearly broken in solution and may or may not be broken in the solid state.

Further examples are the binary and ternary inhibitor adducts of carboxypeptidase, where only the availability of many experimental data and calculations has permitted the refinement of the mechanism., and iron sulfur proteins, where differences are detected between crystal structures and solution structures and between oxidized and reduced species. Equilibria are also detected among various structural isomers, whose interconversion rates may be either fast or slow with respect to the electron transfer rate.

These different conformational arrangements do not require large flexibility of the protein scaffolding, as it may be the case for bioactive peptides; yet, in globular metalloproteins and metalloenzymes these slight differences may involve the active site structure and be crucial for lowering the energetic barrier of one or another distinct mechanistic pathway.

Influence of Surface Charges on Redox Properties in High Potential Iron-Sulfur Proteins

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High potential iron-sulfur proteins constitute a class of iron-sulfur proteins characterized by positive $E°$ values spanning a ~400 mV range (1, 2). They all contain a Fe_4S_4 cluster, that is coordinated by four conserved cysteines. The coordinated sulfur atoms are involved in five conserved hydrogen-bonds with peptide NH protons (3). Preliminary data indicate that a correlation may exists between the number and distribution of charged residues in the various protein and their reduction potentials (4, 5).

With this in mind, we have measured the pH-dependence of the reduction potential through differential pulse voltammetry using a pyrolitic graphite electrode for the high potential iron sulfur proteins (HiPIP) from *R. globiformis, C. vinosum, R. gelatinosus, E. vacuolata* (I and II), *E. halophila* (I and II).

A decrease in reduction potential with pH is invariably observed in the pH range where deprotonation of the imidazolium nitrogen of histidine residue(s) occurs, whereas no pH dependence is observed for the only protein lacking histidines. It appears that surface charges like the His imidazolium groups are capable of influencing the reduction potential despite the known quencing of the electrostatic interactions due to solvent effects.
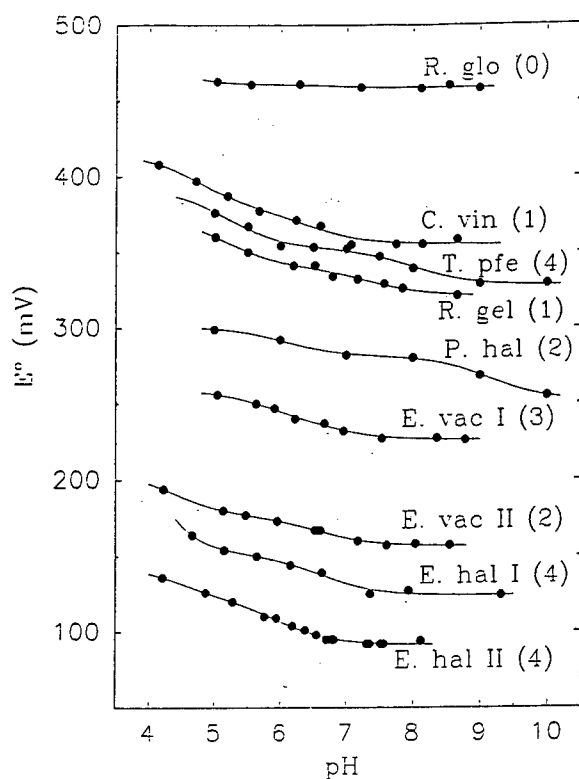
References

1. Cammack, R. (1987) In Iron-Sulfur Protein Research; (H. Matsubara, Y. Katsube, K. Wada, Eds), p 40, Japan Scientific Societies Press / Spriger Verlag, Berlin.
2. Evans, M. C. W. (1982) In Iron-Sulfur Proteins (T. G. Spiro, Ed.), Ch. 6, Wiley, New York.
3. Backes, G., Mino, Y., Loehr, T. M., Meyer, T. E., Cusanovich, M. A., Sweeney, W. V., Adman, E. T., and Sanders-Loehr, J. (1991) J. Am. Chem. Soc. 113, 2055-2064.
4. Rees, D. C. (1985) Proc. Natl. Acad. Sci. USA 82, 3082-3085.
5. Banci, L., Bertini, I., Capozzi, F., Ciurli, S., Gori Savellini, G., and Luchinat, C. (1993) Proceeding of the first Siena-Kyoto Meeting, Kyoto.

Selective Hydrolysis of Dipeptide Mediated on Artificial Enzyme Involving Cobalt(III) Ion

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Molecular recognition in the enzyme-substrate complex selectively appears at or near the active site of the enzyme by a combination of some weak non-covalent interactions. We prepared a new Co(III) complex with a tripodal tetradentate ligand, bis-N,N-carboxymethyl-L-phenylalanine (BCMPA), as a simple enzyme model for the recognition and activation site of the substrate. The coordination characteristics of the Co(bcmpa) complex with amino acid (AA) and dipeptide (DP) were investigated on the basis of ^{1}H-NMR and CD spectroscopies results and X-ray analysis. The hydrolysis of DP in an aqueous solution by this Co(III) complex, as an enzyme model reaction, was also examined.

It has already been revealed that the Co(bcmpa) complex preferentially binds AA in the trans-N configuration rather than in the cis-N form, based on the spectroscopic and structural studies.[1] A similar coordination selectivity was also demonstrated for DP; the amino group of the N-terminal of DP coordinated to the position trans to the nitrogen atom of BCMPA. Such site-specific coordinations with AA and DP were controlled through the interligand non-covalent interactions, such as hydrogen bonding, steric repulsion, and electrostatic interactions. Especially, the DP containing aromatic amino acid at the C-terminal indicated a very interesting interligand interaction for the Co(bcmpa), which is explained by the CH-π interaction between the α-H of BCMPA and aromatic ring of the C-terminal of DP.

Interestingly, the Co(III)-assisted hydrolysis of DP is regulated through the interligand interactions; the DPs without aromatic amino acid at the C-terminal were hydrolyzed, whereas those with them were not at all. This result can be interpreted by the aggregation of the aromatic rings caused by the CH-π interaction, which prevented the approach of OH⁻ species to the amide carbonyl group coordinated to the Co(III) ion. The multi-site interaction appeared on the Co(III) complex is available for the design of an artificial hydrolysis enzyme.

1) K. Jitsukawa, et al., *Inorg. Chim. Acta*, **216**, 249 (1994).

BIOMIMETIC OXYGENATION OF ALKANES BY PHOTOEXCITED IRON-PORPHYRINS

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The preparation and characterization of new catalysts which are models of enzymatic systems is a research field frequently named *"biomimetic catalysis"*. This research focusses both on obtaining information about the *in vivo* mechanism of the enzyme and on building up catalytic systems that exhibit the high selectivity typical of enzymatic processes in mild conditions. Several chemical model systems based on simple metalloporphyrins that mimick monooxygenases have been reported in the last years. On the other hand, only few examples of models of dioxygenases have been investigated.

Photochemical excitation is a suitable mean to induce oxygenation of alkanes through monooxygenase or dioxygenase mechanisms in the presence of iron porphyrins[1]. This biomimetic aspect of the photochemical behaviour of iron-porphyrins has been investigated in homogeneous solution as well as in heterogeneous systems in which the complex is confined on the surface or caged into solid matrices[2].

Herein, we discuss the results obtained using "sterically hyndered" iron porphyrins as photocatalysts for the biomimetic oxygenation of alkanes, at room temperature and at a oxygen pressure of 760 torr. In particular, we comment an interesting dependence on the polarity of the environment in controlling the oxygenation process toward either a biomimetic dioxygenase- or monooxygenase-type mechanism will be discussed.

REFERENCES

1) (a) K.S. Suslick, R.A. Watson, *New J. Chem.*, 633 (1992). (b) D. Mansuy, *Coord. Chem. Rev.* (1993). (c) A. Maldotti, R. Amadelli, C. Bartocci, V. Carassiti, E. Polo, G. Varani, *Coord. Chem. Rev.*, 143 (1993).

2) (a) A. Maldotti, C. Bartocci, R. Amadelli, E., Polo, P. Battioni, D. Mansuy, *J. Chem. Soc. Chem. Comm.*, 1487 (1991). (b) R. Amadelli, M. Bregola, E. Polo, V. Carassiti, A. Maldotti, *J. Chem. Soc. Chem. Comm.*, 1355 (1992).

MECHANISMS OF FORMATION AND DECOMPOSITION OF (GLYCINE)$_2$-Cu^{III}-ALKYL AND OF (GLYCYLGLYCYLGLYCINE)Cu^{III}-ALKYL- IN NEUTRAL AQUEOUS SOLUTIONS.

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The reactions of alkyl free radicals, *i.e.* $\cdot CH_3$; $\cdot CH_2C(CH_3)_2OH$; $\cdot CH_2CO_2^-$, with (glycine)$_2$Copper(II) and with (glycylglycylglycine)Copper(II) in neutral aqueous solutions were studied. Transient complexes of the type (glycine)$_2$Copper^{III}-alkyl and (glycylglycylglycine)Copper^{III}-alkyl$^-$ are formed in all these reactions. The transient complex (glycyl glycylglycine)Cu^{III}-CH$_3^-$ is surprisingly stable. The latter complex has a half life time of *ca* four minutes.

The spectra of the transient complexes as well as the mechanism of their decomposition are reported and discussed. The biological implications of the facile formation of analogous complexes, and their relatively long life time, in free radical processes is discussed.

Characterisation of prismane protein by MCD spectroscopy.

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The sulphate reducing bacteria *Desulfovibrio vulgaris* (Hildenborough) and *Desulfovibrio desulfuricans* contain a putative [6Fe-6S] prismane cluster containing protein (1,4). The *D.vulgaris* protein is monomeric with a molecular mass of 52kDa and contains six irons and six acid labile sulphurs per molecule (2). EPR monitored potentiometric redox titrations performed on the prismane protein have identified four valency states for the iron sulphur cluster (3). These are $[6Fe-6S]^{6+}$, $[6Fe-6S]^{5+}$, $[6Fe-6S]^{4+}$ and $[6Fe-6S]^{3+}$. The fully oxidised +6 state appears to be diamagnetic. The protein as isolated has the cluster in the +5 state, this exhibits EPR spectra characteristic of S=9/2 and S=1/2 ground spin states. Partial reduction of the as isolated protein produces the +4 state of the cluster which is integer spin, possibly S=4. Fully (dithionite) reduced protein contains the cluster in the +3 state, this exhibits an S=1/2 EPR spectrum which shows similarities to that obtained for synthetic model compounds of the prismane cluster.

The structure and redox states of different types of iron sulphur cluster show distinctive MCD spectra. MCD analysis of the as isolated, +5, state of prismane protein shows a weak spectrum dissimilar to that of other iron sulphur proteins. However this spectrum does contain bands characteristic of rubredoxin. The dithionite reduced, +3, state also shows a weak spectrum which can not be identified with a known iron sulphur cluster. The associated magnetisation curves can be fitted to an S=1/2 spin system. The form of the MCD spectrum and magnetisation data for the +4 state are also presented.

1. Hagen, W.R., Pierik, A.J. and Veeger, C. (1989) J. Chem. Soc. Faraday Trans. 1. <u>85</u>, 4083-4090.
2. Pierik, A.J., Wolbert, R.B.G, Mutsaers, P.H.A., Hagen, W.R. and Veeger, C. (1992) Eur. J. Biochem. <u>206</u>, 697-704.
3. Pierik, A.J., Hagen, W.R., Dunham, D. and Sands, D.H. (1992) Eur. J. Biochem. <u>206</u>, 705-719.
4.Berg, van den, W.A.M., Stevens, A.A.M., Verhagen, M.F.J.M., Dongen, van, W.M.A.M. and Hagen, W.R. (1994) Biochim. Biophys. Acta, in press.

Fatalities after overdosing cis-diamminodichloro-platinum(II)

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Summary

Cisplatin is the common name for cis-diamminodichloro-platinum(II). It is generally assumed that DNA is the cellular target responsible for the antitumor activity of the drug. The platinum complex can form both intrastrand and interstrand crosslinks.

Cisplatin has been introduced for the palliative treatment of epithelial malignancies such as testicular and ovarian carcinomas. Major side effects are nausea and vomiting, renal toxicity and bone marrow damage. Besides this known side effects overdosing may lead to life threatening disorders of ventilation and acid base balance. In this case cisplatin overcomes the blood brain barrier.

In the case discussed here a 9-month-old child was to receive cisplatin due to a small tumor at the vaginal posterior wall. The child was treated erromously with 44 mg of cisplatin daily which means a total dose of 400 mg/m². On day 4 tachydyspnoe, tachycardia and fever occurred. Generalised edema were seen also. Renal function was impaired, a severe metabolic acidosis developed. Death by progredient heart failure occurred on day 5 after the beginning of the treatment. Since the body rested in a grave for about 5 weeks on autopsy and subsequent histological examination no unequivocal findings could be raised. The toxicological analysis concentrated on the determination of organ levels of platinum using atomic absorption spectrometry. Relatively high organ levels of platinum were found: liver 21.4 µg/g, kidney 14.2 µg/g, lung 17.4 µg/g, myocard, skeleton muscle 1.5 µg/g organmass (wet weight). No measurable levels were found in brain tissue. Estimations performed on the basis of these concentrations revealed that over 90% of the dose given could be indicated in the whole body. This proofs the impairment of renal function: Normally after 5 days at least 43% of the administered dose are recovered from the urine.

The few cases reported so far in the literature are discussed with respect to the question whether the overdose could have been fatal. □

Binding of small gadolinium chelates to serum proteins enhances their efficacy as contrast agents for MRI.

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Gd-BOPTA/Dimeg is a new paramagnetic contrast agent which differs from the widely used chelate Gd-DTPA/Dimeg in that a methylene proton of one of the terminal acetic acid moieties has been substituted by a benzyloxymethyl side chain. This structural difference has little effect on the relaxivity of the chelate in aqueous solution (r_1=4.39 and 3.77 mM^{-1}s^{-1} for Gd-BOPTA^{2-} and Gd-DTPA^{2-}, respectively, at 20 MHz and 39°C) and a large effect in some biological fluids as for example human plasma (r_1= 9.7 and 4.9 mM^{-1}s^{-1} for Gd-BOPTA^{2-} and Gd-DTPA^{2-}, respectively). Our hypothesis is that the higher relaxivity of Gd-BOPTA^{2-} in plasma is due to binding with serum albumin; this binding is expected to be very weak since it is not measurable in equilibrium dialysis experiments.

Binding of Gd-BOPTA^{2-} to serum proteins was assessed *in vitro* by determining the Nuclear Magnetic Resonance Dispersion (NMRD) profiles for solutions 0.2 mM in Gd-BOPTA^{2-} in plain 50 mM HEPES buffer (I=0.1M) and in the presence of different concentrations (0.44-5.3 mM) of bovine serum albumin (BSA). NMRD profiles have been recorded also for Gd-DTPA^{2-}. Measurements were performed at 25°C on a Koenig-Brown field-cycling relaxometer operating between 0.01 and 50 MHz.

The NMRD profiles of Gd-BOPTA^{2-} showed a hump at high frequency (≈ 20 MHz) which is characteristic of hindered rotational dynamics due to the binding. No such hump was detected in the Gd-DTPA^{2-} profiles. The enhancement factor (ε), which is defined as the ratio between the paramagnetic contribution to the water relaxation rate in the presence and in the absence of the macromolecule, has been calculated at 15 MHz. For Gd-BOPTA^{2-}, the dependence of ε on the protein concentration was that theoretically expected for a weak binding, while ε was constant, about 1, for Gd-DTPA^{2-}, indicating that no interaction occurs. The NMRD profiles of Gd-DTPA^{2-} are simply the sum of the contributions of BSA, of the metal chelate and of water. The hump in the NMRD profiles of Gd-BOPTA^{2-} is due to an extra contribution caused by interactions.

Our *in vitro* studies show that Gd-BOPTA^{2-} weakly binds to BSA with a consequent increase in its relaxivity at imaging field strengths. Since high concentrations of serum albumin have been found in damaged tissues with increased capillary permeability, this mechanism can explain the better performances of Gd-BOPTA^{2-} in delineating myocardial ischemia (1) and brain tumors (2).

1) F.M. Cavagna, P. Marzola, M. Daprà, F. Maggioni, E.Vicinanza, P.M. Castelli, C. de Haën, C. Luchinat, M.F. Wendland, M. Saeed, C . B. Higgins, *Investigative Radiology*, June Supplement 1994.
2) F.M. Cavagna, F. Maggioni, P.M. Castelli, E. Vicinanza, P. Marzola, M. Daprà, E. Felder, Abstract submitted to the "Society of Magnetic Resonance", 2nd meeting, San Francisco (1994).

The redox centres of benzene dioxygenase: assembly, directed mutagenesis and spectroscopy.

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Benzene dioxygenase catalyses the conversion of benzene to *cis*-benzene dihydrodiol in the bacterium *Pseudomonas putida*. The enzyme is a three-component system, comprising a flavoprotein and a ferredoxin, which transfer electrons from NADH to an iron-sulphur protein (ISP) containing a Rieske-type [2Fe-2S] cluster and a catalytic iron centre [1]. The ISP consists of two dissimilar subunits arranged in an $\alpha_2\beta_2$ configuration. Analysis of predicted amino acid sequence indicates that both the [2Fe-2S] cluster and the iron centre are ligated to the α subunit.

We have cloned all of the components [2], and expressed them separately and in combination in *E. coli* and *Pseudomonas* [3]. EPR spectra of cell extracts from the *E. coli* strains expressing the ferredoxin, $\alpha\beta$ subunits in combination and α subunit alone showed that the [2Fe-2S] cluster was fully incorporated. The active enzyme was assembled both *in vitro* and *in vivo* from its constituent subunits. Complementation studies with cell extracts expressing the α and β subunits of an analogous enzyme, toluene dioxygenase, revealed that substrate specificity is conferred by the α subunit.

Well-resolved ENDOR and ESEEM spectra have been obtained from the [2Fe-2S] clusters of the ferredoxin and ISP components, in the cell extracts from recombinant *E. coli* cells. ESEEM spectra of cells expressing either α or $\alpha\beta$ subunits of the ISP were identical to those obtained with ISP purified from *P. putida*. ESEEM spectra showed that the ligand environment of the Rieske-type cluster comprises two strongly-coupled histidine nitrogens. This is in agreement with previous studies which indicate that the [2Fe-2S] cluster is coordinated by two histidines and two cysteines. The cluster in the ferredoxin component showed similar coordination by two nitrogens.

Sequence-specific mutagenesis of the ferredoxin was carried out, to investigate the ligands to the cluster and the nature of its environment. Mutation of histidines 45 and 65 to cysteines, separately or in combination, prevented insertion of the cluster *in vivo*. This suggests that the histidines are ligands to the cluster, and that the protein with four cysteines is not receptive to [2Fe-2S] clusters. Mutation of amino acids in the vicinity of these histidines gave rise to alterations in the physical characteristics of the cluster and reduced enzyme activity.

[1] Mason, J. R. and Cammack, R. (1992) *Ann. Rev. Microbiol.* **46**, 277-305. [2] Tan et al., (1993) Gene, **130**, 33-39. [3] Tan et al., (1994) J. Bacteriol., **176**(9) (in press)

CONFORMATIONAL ASPECTS OF POTASSIUM COMPLEXES WITH CROWN ETHERS

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Macrocyclic polyethers represent a class of compounds of significant interest in coordination chemistry from different points of view ranging from synthetic to structural and analytical aspects. Part of this interest is due to studies of the role of alkali metal ions (especially sodium and potassium) in the biological systems and also of mechanism for transporting through the cell membranes. It was shown that macrocyclic polyethers provide good model systems for biochemical membrane transport agents. Their transport behaviour was found to be largely dependent on the nature of the counterion which accompanied the cation-macrocyclic polyethers complex. The stability of the complexes is strongly affected also by the size of cation.

Our investigation is a part of a systematic study on the complexes of alkali metals with crown ethers in order to determine the conformation of the macrocyclic ligands taking into consideration the various alkali cations and also the steric hindrance effects of alkyl substituents on the phenyl rings on the crown.

We report here the crystal structure of two potassium tetraphenylborate complexes: **(1)** with dibenzo-18-crown-6 and **(2)** with bis(3,5-di-tert-butylbenzo-18-crown-6. It is interesting that the conformation of the two macrocyclic rings are very different. Also the coordination of the potassium ion is different. In the complex **(1)** K is coordinated by six O polyether atoms and by O atom from the methanole (solvent)., while in the complex **(2)** cation is coordinated by the six O atoms from the macrocycle, but there is a kink in the ring as one O atom assumes an apical position above the other five.

METAL IMIDAZOLE INTERACTIONS: DEVELOPMENTS IN THE CHEMISTRY OF VERSATILE BIOLOGICAL LIGANDS.

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A series of Cu(I) and Cu(II) complexes of the ligands **L1**, **L2** and **L3** have been synthesised. These have been used to assess the potential of bidentate imidazoles as a means of achieving our major goal of preparing imidazole complexes that mimic the electronic, spectroscopic and geometric features and/or reactivity of active sites in metal containing proteins and enzymes.

The strategy of using sterically hindered chelates has yielded Cu(I) and Cu(II) complexes with equivalent ligation, low coordination number and, as X-ray crystallography on $[(L1)_2Cu][PF_6]$ and $[(L1)_2Cu][BF_4]_2$ has shown, controlled stereochemistry. Both these structures possess Cu ions in similar environments that may be described as distorted tetrahedral. The Cu(I) and Cu(II) complexes exhibit charge-transfer absorptions in the UV/vis region and EPR parameters characteristic of distorted metal sites.

Electrochemistry in CH_2Cl_2 has revealed a reversible Cu(II)/Cu(I) couple at 0.800V vs SSCE for the $[(L1)_2Cu]^{n+}$ complexes. Reversibility was anticipated on the basis of the equivalent ligation in the oxidised and reduced forms of the complex. Other examples of such systems are rare[1] and include the type I copper containing protein, plastocyanin[2].

By using sterically more hindered ligands, such as **L3**, we have been able to prepare the bis-imidazole complexes $(L3)Cu(NO_3)_2$ and $(L3)CuBr_2$. We are currently assessing the reactivity of these complexes toward other biologically relevant ligands containing thiol, thioether and carboxylate functionalities.

1. J. A. Goodwin, D. M. Stanbury, L. J. Wilson, C. W. Eigenbrot and W. R. Scheidt, *J. Am. Chem. Soc.*, 1987, **109**, 2979.; S. Knapp, T. P. Keenan, X. Zhang, R. Fikar, J. A. Potenza and H. J. Schugar, *J. Am. Chem. Soc.*, 1990, **112**, 3452.

2. J. M. Guss, P. R. Harrowell, M. Murata, V. A. Norris and H. C. Freeman, *J. Mol. Biol.*, 1986, **192**, 361.
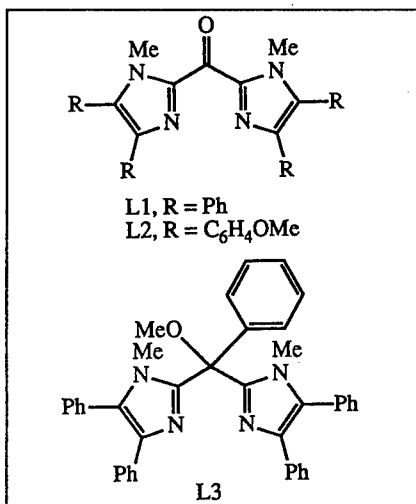
In vivo incorporation of Fe, Mn, Cu or Co into a superoxide dismutase: Was
the original function of the enzyme in anaerobes the complexation of trace
metals in excess?

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Superoxide dismutases (SOD) are regarded as one of the defensing enzymes
against oxygen toxicity. Anaerobic bacteria, however, also possess high
amounts of SODs suggesting that the enzyme originally had another function.
Propionibacterium freudenreichii sp. shermanii builds a single constitutive
SOD, which represents about 1%-2% of the total cytoplasmatic protein and
has iron, or if the bacteria are grown on an iron depleted medium, manga-
nese incorporated into the same protein moiety. Both SODs possess similar
enzymatic activity (1). Depending upon metal supplementation to the medium
also copper (2) or cobalt can be incorporated by the bacteria into the SOD
in stochiometric amounts leading to an inactive form. Decisive for the
incorporation seems to be the intracellular concentration of trace metals.
In an iron depleted medium, manganese is enriched by the bacteria and
mainly incorporated into the SOD whereas other trace metals are not affect-
ed. If the medium is free of iron and manganese, intracellular copper in-
creases and is preferably incorporated into the SOD as well as cobalt is
enriched and incorporation into the SOD in a medium also lacking copper.
Both superoxide dismutases are unable to catalyze the dismutation of su-
peroxide. These observations lead to the hypothesis that the original
function of SOD was the complexation of intracellular trace metals present
in excess.

1. Meier, B., Barra, D., Bossa, F., Calabrese, L., Rotilio, G. (1982) J.
Biol. Chem. 257, 13977-13980

2. Meier, B., Sehn, A.P., Schininà, Barra, D. (1994) Eur. J. Biochem. 219,
463-468

CATION SPECIFICITY OF F-TYPE ATPASES (ATP SYNTHASES)

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F-type ATPases are multimeric enzymes present in all energy transducing membranes of photosynthetic or respiratory apparatuses. Their physiological function consists in the synthesis of ATP from ADP and orthophosphate, energetically driven by a transmembrane flux of protons, or, reversibly, in the hydrolysis of ATP coupled to the active translocation of protons. All F-ATPases are similar in their basic structure: they are formed by an extrinsic sector (F_1), in which five polypeptides are present in a $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry, and a transmembrane sector (F_o) with three polypeptides in a ab_2c_{9-12} stoichiometry. While F_o forms a proton conductive device across the membrane, F_1 contains three catalytic sites.

The enzyme can catalyze the hydrolysis of ATP coupled to proton transport; substrate of this reaction if the complex of ATP with divalent cations, being Mg^{2+} the physiological one. Among the other cations that can substitute Mg^{2+} (Zn^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} and Ca^{2+}) only Ca^{2+} confers special characteristic to the enzyme, as follows: a) the hydrolysis of Ca-ATP is catalyzed as well as that of Mg-ATP by the membrane bound enzyme, but a Ca-specific activity can be often induced in the isolated F_1 sector by various treatments; b) although Ca-ATP and Mg-ATP compete for the same catalytic site, only the Mg-dependent ATPase is activated by the transmembrane $\Delta\mu_{H^+}$ when the enzyme is membrane bound; c) while Mg-ATP hydrolysis is coupled to the translocation of protons, Ca-ATP hydrolysis is not and Ca-ATP behaves as a competitive inhibitor for proton translocation; Mg-ADP, but not Ca-ADP is a substrate for ATP synthesis.

A SYNTHETIC MIMIC OF THE Hg(II)-BINDING SITE
OF THE METALLOREGULATORY PROTEIN MerR

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Metalloregulatory proteins play a pronounced role in biological systems[1]. Some control the levels of essential metal ions within a narrow window, others initiate disposal of toxic elements. An example of the latter is the metalloregulatory protein MerR, which initiates the elimination of toxic Hg(II). MerR responds to Hg(II) by a conformational change, which initiates transcriptional activation of DNA and consequently synthesis of mercuric reductase, which reduces Hg(II) to volatile, elementary Hg.

Here we describe an all-artificial Hg(II)-binder that reproduces the structure and properties of the ion-binding site of natural MerR. The synthetic molecule has been assembled from a C3-symmetric molecule as anchor and three extending cysteine residues as binding sites. The cysteine residues of this binder have been substituted by naphthyl residues which impart to the molecule two significant advantages: they stabilize the binder towards oxidation and serve as fluorescent markers for ready detection.

Relying on ^{1}H and ^{13}C- NMR we demonstrate that the synthetic Hg(II)-complex adopts a single configuration of C3-symmetry, similar to the coordination geometry suggested for the Hg(II)-complex of MerR. We further show, that the binder is stable to oxidation and that binding of Hg(II) manifests itself by fluorescence quenching. In addition, the synthetic binder, similar to MerR, effectively binds Hg(II) in preference to other heavy metals such as Cd(II) and Pb(II). The relevance of these findings for future applications of the novel Hg(II) binder will also be indicated.

1) Thomas V. O'Halloran, "Transition Metals in Control of Gene Expression", *Science, 261,* 751 **(1993).**

BIOMIMETIC IRON(III) SCAVENGERS AS
POTENTIAL ANTIMALARIAL AGENTS

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In an attempt to develop antimalarial agents that are effective against chloroquine sensitive as well as chloroquine resistant strains of Plasmodium falciparum, we designed and synthesized iron(III) carriers that selectively inhibit parasite growth by depriving it of essential iron(III).

The design of these compounds relies on natural iron carriers, siderophores, such as ferrichrome as guiding models. The binding cavities of the natural compounds were reproduced to achieve favorable iron(III) binding properties, but their hydrophilic envelopes replaced by varying hydrophilicity to facilitate penetration into infected red blood cells.

In vitro assays of antimalarial activity showed inhibition of parasite growth that correlates with iron(III) binding properties and hydrophobicity of the compounds[1] and *in vivo* experiments in infected mice revealed suppression of parasitemia[2]. The possibility of irreversible growth inhibition of parasites with some of the chelators, but reversible inhibition and recovery of mammalian cells will be discussed[3] as well as the use of fluorescent labels for monitoring iron trafficking[4].

1- A. Shanzer, J. Libman, S. Lytton, H. Glickstein, I. Cabantchik; **Proc. Natl. Acad. Sci, 88,** 6585 (1991)

2- S. Lytton, M. Loyevsky, B. Mester, J. Libman, I. Landau, A. Shanzer, I. Cabantchik; **Am. J. Hematology, 43,** 217 (1993)

3- S. Lytton, B. Mester,.J. Libman, A. Shanzer,I. Cabantchik; **Blood** (in press)

4- S. Lytton, B. Mester, J. Libman, A. Shanzer and I. Cabantchik, **Anal. Biochem., 205,** 326- 333(1992)

Metal-Modified Guanine, Cytosine Base Pair

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Although *trans*-DDP (= *trans* [(NH$_3$)$_2$PtCl$_2$]) does not, unlike its *cis*-isomer, exhibit antitumor activity, some interest has recently focussed on *trans*-DDP nucleobase complexes, especially on those containing two different nucleobases which can be regarded as "metal modified base pairs". A large variety of Hoogsteen- and Watson-Crick type metal bridged nucleobase pairs as well as non-complementary ones has been prepared so far [1,2,3], including the Hoogsteen analogue of the guanine, cytosine pair [2].

The Watson-Crick analogue of this base pair has been difficult to prepare due to preferential Pt binding to the kinetically favoured N7 position. Using 7,9-dimethylguanine (7,9-DMG) instead of 9-R-guanine, a compound of composition *trans*-[(NH$_3$)$_2$Pt(1-Mec)(7,9-DMG)] (ClO$_4$)$_2$*2 H$_2$O, (1), has now been isolated and characterized by ^{1}H-NMR, vibrational spectroscopy and elemental analysis. Crystals suitable for X-ray analysis are presently prepared.

With intramolecular H bonding between bases in (1) no longer possible, it should be interesting to find out wether, as in the platinated adenine, thymine Watson-Crick analogue [2], H$_2$O is involved in keeping the bases planar.

Fig. 1: expected structure of (1)
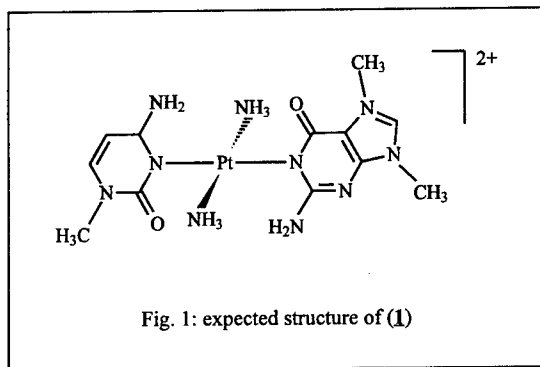
A dark green heteronuclear derivative of (1) of composition *trans*-[(NH$_3$)$_2$Pt,Cu(1-MeC)(7,9-DMG)](ClO$_4$)$_2$*n H$_2$O has been obtained by reacting (1) with one equivalent of CuSO$_4$ in concentrated ammonia solution.

Lit.:

[1]   A. P. Hitchcock, C. J. L. Lock, W. M. C. Pratt, B. Lippert in "Platinum, Gold and other Metal Chemotherapeutical agents", S. J. Lippard (ed.), ACS Symposium Series 209, Am. Chem. Soc. Washington DC 1983, p. 209

[2]   O. Krizanovic, M. Sabat, R. Beyerle-Pfnür, B. Lippert, J. Am. Chem. Soc. 115, (1993) 5538

[3]   S. Menzer, M. Sabat, B. Lippert, J. Am.Chem. Soc. 114 (1992), 4645

Resonance Raman Spectroscopy of Recombinant Human Tyrosine Hydroxylase

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The human tyrosine hydroxylase isozyme-1 (hTH1) was expressed in *E.coli*, purified as the apoenzyme and reconstituted with iron. The resonance Raman spectra of hTH1 complexed with dopamine, noradrenaline, tyramine and catechol have been studied and compared to those obtained for TH isolated from bovine adrenals or rat phaeochromocytoma tissue. Using specifiquely ^{18}O labeled dopamine in the 3-position or both 3- and 4-hydroxy positions, we have been able to assign unambiguously the origin of the vibration bands : the band at 631 cm^{-1} involves the oxygen in the 4 position ; the band at 592 cm^{-1} involves the oxygen in the 3 position and the band around 528 cm^{-1} is shifted by both, suggesting a chelated mode vibration ; almost no shifts of the 1275 and 1320 cm^{-1} bands were observed, showing that those two bands involve essentially ring vibrations of the catecholate motif. The spectrum of the d_6-catechol-hTH1 complex confirm this assignment since deuteration leads to a dramatic shift of the band at 1275 cm^{-1} to 1200 cm^{-1} and the complete disappearance of the band at 1320 cm^{-1}. The resonance Raman spectra of the ^{54}Fe, ^{56}Fe or ^{57}Fe isotope containing enzymes complexed with dopamine are virtually identical, showing that the component of the iron in the ≈ 600 cm^{-1} vibrations is too small to be observed. These results provide a better understanding of the Raman properties of iron-catecholate complexes in other metalloproteins and model compounds.

NMR-study of base-selective interaction of the nickel(II) ion to the DNA-oligomere 5´-d(GACGGCCGTC)

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Several studies has shown that nickel act as a carcinogenic specimen. In fact - the collective evidence derived from in vitro genotoxity, animal carcinogenity and erythropoiesis studies can be interpreted to indicate that the Ni(II) ion is the putative agent - the ultimate carcinogen[1].

$NiCl_2$ has been chosen as the compound for the titration with DNA. This because the chlorine ion has no harmful side effects and because of it's solubility in water. In water nickel(II) forms octahedral complexes which are paramagnetic. There is no transition to square-planar complexes(which are diamagnetic) during interaction with nucleic bases or phosphates[2].

Because of the paramagnetic behaviour of the Ni^{2+} ion, very low concentrations have been used(in the range 10^{-6} M). Paramagnetic species produces line broadening in NMR-spectra, but when it is titrated quantitatively into the NMR-tube certain peaks in the NMR-spectra will broaden. These peaks, when assigned, give information about binding-sites in the molecule investigated.

The background for choosing the palindromic decamere 5´-d(GACGGCCGTC) is the base-sequences GG and GA. Previous studies have shown a special metal affinity or selectivity to these patterns[3].

The study has been based on 1D ^1H and 2D NMR-techniques. Peak-assignment has been done with 1D and COSY. Redfield pulse-sequences have been used to suppress the dominant water signal.

2D NOESY is the technique used to give structural information about the decamere.

[1]E. Nieboer, F. E. Rossetto and C. R. Menon in "Metal ions in biological systems", vol. 23, 10.5.2, Marcel Dekker Inc., New York (1988)

[2]R. B. Martin in "Metals in biological systems", vol. 23, 8, Marcel Dekker Inc., New York (1988)

[3]N. Å. Frøystein, J. T. Davis, B. R. Reid and E. Sletten, *Acta Chem. Scand.* 47, 649-657 (1993)

NMR and X-Ray Characterization of Nickel Azrurin from
Pseudomonas aeruginosa

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Pseudomonas aeruginosa (*Pae*) Azurin is a blue copper protein (MW 14 kDa) which probably participates in the electron transfer process when the bacterium grows under denitrifying conditions. The copper atom is coordinated to two imidazole nitrogens ($N^{\delta 1}$ of His46 and His117), two sulfur atoms ($S^{\gamma}$of Cys112 and S^{δ} of Met121) and to the carbonyl group of Gly45, in a distorted trigonal bipyramidal geometry. Replacement of copper by other transition metal ions allows the study of the active site without modifying the overall structure of the protein. We are performing the characterization of the nickel derivative by applying both NMR spectroscopy[1] and X-Ray diffraction.

From our X-Ray data, nickel coordination in *Pae* azurin remains essentially the same as in the zinc substituted protein[2], showing a slight displacement of the metal towards the carbonyl group of Gly45, which is now 0.5 Å nearer to the metal ion than in the native azurin[3]. Furthermore, the S^{δ} of Met-121 probably is non-bonded to the metal ion since the Ni(II)---Met121S^{δ} distance is as large as 3.35 Å. These results are not in agreement with the conclusions of the ^{1}H NMR studies on Ni(II)-azurin[1]. Signals assigned to the Met121 protons show large isotropic shifts and so, in principle, we have to admit the existence of contact contribution. We are performing the specific assignment of protons belonging to residues not directly coordinated to the paramagnetic metal ion by 2D NMR techniques with the aim of determining the magnetic anisotropy tensor in the nickel(II)-azurin and the effect that this magnetic anisotropy produces in the protons around the metal ion. This will allow us to evaluate the magnitude of the pseudocontact contribution in this metalloprotein and so to separate contact and dipolar contributions for the protons of the coordinated residues. Similar studies are being carried out with the cobalt derivative.

[1] J.M. Moratal, J. Salgado, A. Donaire, H.R. Jiménez, J. Castells, M.J. Martínez. *Magn. Res. in Chem.*, **1993**, *31*, 41-46.

[2] H. Nar, R. Huber, A. Messerschmidt, A.C. Filippou, M. Barth, M. Jaquinod, M. van de kamp, G.W. Canters. *Eur. J. Biochem.*, **1992**, *205*, 1123-1129.

[3] H. Nar, A. Messerschmidt, R. Huber, M. van de Kamp, G.W. Canters. *J. Mol. Biol.* **1991**, *221*, 765-772.

IRON(III) SUPEROXIDE DISMUTASE FROM *E. COLI*: ELECTRONIC PROPERTIES OF THE ACTIVE SITE FROM EPR AND MAGNETIC SUSCEPTIBILITY STUDIES

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Iron(III) superoxide dismutase active site is characterized by a trigonal bipyramidal arrangement around the metal ion with four protein ligands and possibly a water molecule (1).

Magnetic susceptibility (or magnetization) and EPR studies are efficient in probing electronic structure and especially the ground state structure which is important with respect to the possible mechanisms. We have applied a combination of the two techniques in order to evaluate the ground state zero-field splitting which is strongly related to the symmetry of the metal center and its chemical environment.

The experiments have been performed on the enzyme from *E. Coli*, following previously developed methods(2). The effective magnetic moment has a constant value of 5.85 (Bohr magnetons) over a large range of temperature which is an indication of a S=5/2 ground state with a small spin quantum mixing. The EPR spectrum reveals three intense signals typical of the transitions within the intermediate Kramers doublet of the 5/2 spin system in rhombic symmetry(3). The fit of the corresponding effective g values to theoretical equations(4) gives an estimation of the degree of rhombicity $E/D= 0.25$. From the temperature dependence of their intensities, two weak signals at low field have been associated to the ground doublet and the second excited one. The energy gaps within the three doublets correspond to the value of the zero field splitting parameter equal to $D = -2cm^{-1}$, associated to the $|\pm 5/2>$ ground Kramers doublet. These properties have been interpreted in terms of ligand field theory (5).

(1) (a) W.C.Stallings, T.B.Powers, K.A.Pattridge, J.A.Fee , M. Ludwig, Proc. Natl. Acad Sci USA 80, 3884-3888 (1983). (b) B.L.Stoddard, P.L.Howell, D.Ringe, G.A.Petsko, Biochemistry 29, 8885-8893 (1990)

(2) I. Morgenstern-Badarau, D.Cocco, A.Desideri, G.Rotilio, J.Jordanov, N.Dupré, J. Am. Chem. Soc. 108, 300-302, 1986

(3) (a) K.Puget, A.M.Michelson, Biochimie 56, 1255-1267 (1974). (b) T.O.Slykhouse, J.A.Fee, J. Biol. Chem. 251, 5472-5477 (1976)

(4) H.H.Wickman, M.P.Klein, D.A.Shirley, J. Chem. Phys. 42, 2113-2117 (1965)

(5) X.Y.Kuang, I.Morgenstern-Badarau, M.C.Rodriguez, Phys.Rev.B 48, 6676-6679 (1993)

Polymetallic Biomodels Containing Supported
or Unsupported Bridging Groups

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The synthesis, structure, magnetism and electrochemistry will be described for binuclear complexes of Mn^{II}, Fe^{III} and Cu^{II} containing both the tris(1-pyrazolyl)methane ligand[1,2] as a tridentate facial 'blocker' and various unsupported bridging groups such as $\mu-O^{2-}$, $\mu-OH^-$, $\mu-RCO_2^-$. These complexes provide useful models for the active sites found in Mn-catalase, oxo-iron and Type 3 Cu proteins. They will be compared with related models formed from tris(1-pyrazolyl)hydridoborate ligands.[3]

Progress in the design and synthesis of new tetranuclear Ni_4 and Cu_4 'pair-of-dimer' complexes, similar to those described earlier,[4] will be presented. These compounds contain supported bridging groups held within polyfunctional chelating ligands.[5]

References
[1] Trofimenko, S., *Progr. Inorg. Chem.*, 1986, **34**, 115.
[2] Fallon, G. D., Knight, S., Moubaraki, B., Murray, K. S., Skelton, B. W. and White, A. H., *J. Inorg. Biochem.*, 1993, **51**, 504.
[3] Kitajima, N., and Moro-Oka, Y., *J. Chem. Soc. Dalton Trans.*, 1993, 2665.
[4] Kruger, P. E., Fallon, G. D., Moubaraki, B. and Murray, K. S., *J. Chem. Soc. Chem. Commun.*, 1992, 1726.
[5] Kruger, P. E., Fallon, G. D., Moubaraki, B. and Murray, K. S., *J. Inorg. Biochem.* 1993, **51**, 473.

Spectroscopic characterization of proteins containing multi-iron centers

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Recently, a number of proteins isolated from anaerobic sulfate reducers have been shown to contain novel combinations of iron centers. The unusual and diversified class of metal centers found in these proteins offer a unique opportunity to explore their magnetic and spectroscopic properties.

We will focus on three proteins with special relevance to their biochemical, EPR and Mössbauer spectroscopic characteristics in order to unreveal the structural and redox properties of their active sites. These proteins display the presence of redox metal centers with metal compositions spanning from mono, through bi to hexa nuclearity as listed below:

i) a protein designated Rubrerythrin (Rr) is a trivial name given to this non-heme iron protein containing rubredoxin-type and hemerythrin-type centers (the designation is a contraction of Rubredoxin and Hemerytrin);

ii) Desulfoferrodoxin (Dfx) is a different protein containing a desulforedoxin (Dx) center (a distorted rubredoxin type center found in the protein isolated from *Desulfovibrio gigas*) and a new mono-iron center bound mainly to nitrogen and/or oxygen ligands;

iii) a novel [Fe-S] protein in which the metal centers are found to manifest in the form of a 6Fe cluster of unknown structure.

The physiologic roles of these proteins are not yet known, but the study of their spectroscopic properties and comparison with other known enzymes, will contribute to the search of their biological functions. Moreover, these new proteins offer the unique opportunity to explore the meaning of the association between simple metallic centers (i.e., rubredoxin and desulforedoxin) forming more complex arrangments, as well as opening new insights in the study of the roles of iron-sulfur centers in biology.

4-HYDROXYBUTYRYL-COA DEHYDRATASE: THE ROLES OF FAD AND IRON-SULFUR CLUSTERS IN A NON-REDOX REACTION

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4-Hydroxybutyryl-CoA dehydratase from *Clostridium aminobutyricum* catalyses the dehydration of 4-hydroxybutyryl-CoA to crotonyl-CoA. The enzyme has recently been purified and is described as a homotetramer with two molecules of FAD and about 16 molecules of both non-heme iron and sulfur per holoprotein (Scherf and Buckel, 1993). The reaction is chemically interesting in that a non-activated C-H bond has to be cleaved and despite the presence of typical redox-cofactors, the overall reaction does not involve the transfer of electrons.

Hence, an aconitase-type mechanism (Beinert and Kennedy, 1989) has been proposed in which the iron-sulfur clusters are not redox active, but rather assume a substrate-polarizing role. Catalysis may be initiated by a two electron oxidation of the substrate to give 4-hydroxycrotonyl-CoA and reduced flavin. The following step could be a hydride attack either at the C2-position (S_N2' reaction) to give vinylacetyl-CoA followed by an isomerization to crotonyl-CoA. Alternatively, the hydride could attack at C4 to give crotonyl-CoA directly. In either case it is proposed that the leaving group is polarized through interaction with the iron-sulfur cluster.

The enzyme has been reduced by various methods to investigate the relative redox potentials of the cofactors. Upon photoreduction in the absence of a mediator such as a viologen, only the flavin undergoes reduction, from oxidized to semiquinone and fully reduced species. Interestingly, there is no reduction of the Fe-S cluster. The semiquinone is generated only kinetically but not stabilized: in the presence of a viologen, photoreduction procedes directly to the fully reduced species. This argues against a radical mechanism which has been suggested (Willadsen and Buckel, 1990).

Photoreduction in the presence of methyl viologen, titration with dithionite and redox titration with the electron generating system described by Massey (Massey, 1993) leads to reduction of both the flavin and the Fe-S cluster. In all cases, the Fe-S cluster remained oxidized until completion of the flavin reduction. Redox reaction in the presence of indigo disulfonate (-116 mV) indicated a redox potential for the flavin in the order of -150 mV, whereas the Fe-S center seemed to equilibrate with reduced methyl viologen (-440 mV). Further investigations are in progress for exact determination of the redox potentials.

It can be concluded, however, that the redox potential for the FAD is clearly higher than that of the Fe-S cluster. This is consistent with the proposed reaction mechanism, in which the flavin becomes reduced during turnover without a subsequent redox equilibration with the Fe-S centers.

Beinert , H. and Kennedy, M.C. (1989) *Eur. J. Biochem.* **186**, 5-15.
Massey, V. (1991) in *Flavins and Flavoproteins* (Curti, B., Roncho, S., & Zanetti, G., Eds.), 59-66, Walter de Gruyter, Berlin, New York.
Scherf, U. and Buckel, W. (1993) *Eur. J. Biochem.* **215**, 421-429.
Willadsen, P. and Buckel, W. (1990) *FEMS Microbiol. Lett.* **70**, 187-192.

"What do we know about the biosynthesis of the big metal cluster of nitrogenase ?

Information from genetics and *in-vivo* spectroscopy"

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The nitrogenases, the conventional as well as the alternative ones, which are the important enzyme systems for nitrogen fixation, consist of two oxygen-sensitive metalloproteins, the MFe protein (M = Fe, V, Mo) and the Fe protein. As the heterometal clusters play a key role in the process, information about their properties and biosynthesis seems to be of significance. The type of cofactor (FeMoco, FeVco, FeFeco) and also the protein environment of these clusters influence the activity and the substrate selectivity of the nitrogenases. Now we have some preliminary information about the kind of cluster biosynthesis, an information which seems to be important as chemists were not able to synthesize the clusters in thousands of experiments. We concentrate now on the study of the Mo processing by TDPAC spectroscopy using bacteria strains lacking genes for FeMoco-synthesis. We are, for instance, in the meantime able to detect an *in-vivo* cluster exchange.

KINETICS OF OXIDATION OF o-DIANISIDINE BY HYDROGEN PEROXIDE IN THE PRESENCE OF ANTIBODY COMPLEXES OF IRON(III) COPROPORPHYRIN

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Monoclonal antibodies against metalloporphyrins have recently received considerable attention as models for the study of apoprotein - prosthetic group interactions and new enzyme-like catalysts. Data on the binding of anti-porphyrin antibodies D5E3 and D3F5 demonstrates structural similarity in the location of metalloporphyrins in peroxidases and antibodies. Binding constants were sensitive to the metal and especially to the nature and arrangement of substituents at the periphery of the macrocyclic ligand. These results showed the binding site of antibodies to be complementary to the ligand and possess a donor group bound to the metal in the antibody-hapten complex. Equilibrium constants reached 10^{-11} M.

The complex of Fe(III) coproporphyrin I (FeCPI) with D5E3 was studied as artificial peroxidase using o-dianisidine as a substrate. The dependence of initial rates of the reaction on substrate concen-trations obeyed Michaelis-Menten kinetics and revealed a substrate activation at high concentrations of o-dianisidine. At saturation with respect to antibody, the catalytic rate constants (k_c) for antibody bound FeCPI exceed k_c for free FeCPI by 2-3 times. From the analysis of the k_c dependence on H_2O_2 concentration for FeCPI -antibody complex it can be supposed, that interaction with H_2O_2 is the rate limiting step for FeCPI-antibody system.

Tetraheme cytochromes associated with photosynthetic reaction centres: A model system for the study of interactions and electron transfer within multiheme proteins

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Most species of purple bacteria contain a tetrahemic redox protein associated with their photosynthetic reaction centre (RC) and serving as electron donor to the photooxidized pigment of the RC. The tetraheme subunits of some species, i.e. *C. vinosum, R. gelatinosus* and *R. viridis* have been studied in detail (for a review, see [1]). The presence of a high resolution X-ray structure for the subunit from *R. viridis* [2] and of the primary sequences for the respective subunits from a number of species allows the prediction of structural models for the purple bacterial tetraheme subunits [3]. In most tetraheme subunits, the four hemes fall into two distinct groups with respect to their redox midpoint potential, i.e. one pair of hemes titrating around 300 to 400 mV (the "high potential pair") and another pair having Em-values in the range between -100 mV and +150 mV (the "low potential pair"). The individual hemes are aligned in a roughly linear row with an alternating sequence of high and low potential hemes. Electrostatic calculations based on the crystal structure of the *R. viridis* subunit predict electrostatic interaction energies as high as 100 mV between pairs of neighbouring hemes [4]. In fact, in some of the systems, electrostatic interactions of the expected magnitude can be observed experimentally [3,6,5]. These interactions between pairs of hemes seem to be strongly modulated by the surrounding solvent [1,3]. Taking the influence of the dielectric medium on electrostatic interactions into account, apparent discrepancies between the results obtained on the purple bacterial tetraheme subunits and the tetrahemic cytochrome c3 from sulphate reducing bacteria can be rationalized.

[1] Nitschke & Dracheva (1994) in Anoxygenic Photosynthetic Bacteria
      (Blankenship, Madigan & Bauer, eds.), Kluwer, in press
[2] Deisenhofer, Epp, Miki, Huber & Michel (1995) Nature 318,618
[3] Nitschke & Rutherford (1994) Biochem.Soc.Trans., in press
[4] Gunner & Honig (1991) PNAS USA 88, 9151
[5] Ortega & Mathis (1993) Biochemistry 32, 1141
[6] Nitschke, Jubault-Bregler & Rutherford (1993) Biochemistry 32, 8871

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THE POLLUTION OF HEAVY METALS FOR SELECTED TOWNS
OF BESKID ŚLĄSKI

Investigation heavy metals in water, air and soil were provided in selected towns of Beskid Śląski (non-industrrial part of southern Poland). During 5 year period (1990-1994) we determined the concentrations of heavy metals in towns: Ustroń (n=90samples,), Wisła (n=350 samples), Szczyrk (n=350 samples), Istebna (n=350 samples), Koniaków (n=350 samples). The ecological estimation is based on analysis of contamination coefficients in air, geochemical cumulation coefficients in soil, the enrichment coefficients and exposure dose for heavy metals. The influence of following pollution sources: -linear sources (motor way, traction of railway), -surface sources (long term range emission), -coal -fired furnaces in the habitable houses is examined. The investigation take into consideration street traffic intensity.

The suspended dust samples were collected for 1 hour using a Staplex dust counter with the flow rate of 16-35 m^3/h. Glass filters (TFGAF-810 type manufactured by U.S.A Staplex Company) with the diameter of 10 cm and 99.95% effectiveness for particle size larger than 0.3µm were used. The dust was mineralised by the mixture of hydrofluoric and nitric acids, while the soil samples were digested in the mixture of hydrofluoric and perchloric acids. Atomic absorption spectroscopy (AAS manufactured by Carl Zeiss Jena) method was used to determine the concentrations of following metals: Pb, Cd, Mn, Cr, Co, Cu, Ni and Zn (the detection limits are about Cu-0.003, Pb-0.07, Zn-0.005 [µg/ml]) .

The values of characteristic parameters indicate to high contamination of the air and soil near linear emission sources. People living near roadway and railway in the non-industry towns inhale similar heavy metal dose like people living in typical industrial town. The dose of exposure for the sum of heavy metals (Mn, Cd, Pb, Cu, Cr, Co, Ni, Zn) during one year and for a man living near the roadway in Wisła is equal 0.17 µg/man and is more than 3 times higher comparing to the "clean" region of Wisła -town.

THE REACTION OF HYDROXYUREA WITH THE IRON/TYROSYL RADICAL CENTER FROM HERPES SIMPLEX VIRUS TYPE 1 RIBONUCLEOTIDE REDUCTASE R2 SUBUNIT.

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Ribonucleotide reductase is a key enzyme for DNA synthesis in living cells. It catalyzes the reduction of ribonucleotides to the corresponding deoxyribonucleotides, which is necessary for DNA replication. The enzyme consists of two nonidentical subunits, proteins R1 and R2. Each subunit is composed of two identical polypeptide chains. The R1 subunit contains redox-active thiol groups and binds substrates and allosteric effectors. Each polypeptide chain in the R2 subunit contains a pair of μ-oxo-bridged ferric ions and a tyrosyl free radical.

The light absorbance bands of HSV1 R2 protein were characterized by first derivative light absorption spectroscopy. The iron bands are located at 327 nm and 377 nm and the tyrosyl free radical bands are located at 397 nm and 417 nm. Light absorption was used to probe the reactions of the iron/tyrosyl free radical from the HSV1 R2 protein with hydroxyurea, a potent inhibitor of iron containing ribonucleotide reductases. In the R2 protein from mouse hydroxyurea destroys the specific absorbance bands of both the iron center and the tyrosyl radical on a similar time scale and this is accompanied by release of iron from the R2 protein. The second order rate constant for the mouse R2 tyrosyl radical is 1.45 $M^{-1}s^{-1}$ at 25° C. Also in the R2 protein from HSV1 hydroxyurea destroys the specific absorbance bands for both the tyrosyl radical and the iron center. The loss of iron can be followed by adding hydroxyurea together with desferrioxamine, a potent iron chelator which forms a ferrioxamine complex with specific light absorption at 430 nm. After 30 min incubation of 50 µM R2 protein (polypeptide concentration) from HSV1 with 0.5 mM hydroxyurea and 0.5 mM desferrioxamine at 15° C the light absorption at 430 nm corresponds to 150 µM ferrioxamine complex. Assuming 2 iron ions per polypeptide chain (2 X 50 µM), this result indicates that the iron release from the R2 protein is complete, probably also including some iron (1 X 50 µM) not bound in the dimeric iron site.

MOLECULAR RECOGNITION IN IRON, COPPER, ZINC AND MERCURY RESPONSIVE METALLOREGULATORY PROTEINS

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Several classes of metalloproteins are involved in signal responsive translation or transcription of genes. Catalytic or structural roles for Mg(II) and Zn(II) have been established for most polymerases and for a variety of transcription factors such as TFIIIA, SP1 and Gal4. On the other hand, the metalloregulatory proteins are a functionally distinct class of metalloproteins that translate signals such as changes in metal ion concentration into changes in gene expression. These DNA or RNA-binding proteins are intracellular metal ion receptors that switch on or off specific genes. We are studying the molecular basis of metal ion recognition by the mercuric ion receptor MerR, the copper sensor pCoR/pCoS and the iron sensor Fur and testing whether the broader class of zinc finger proteins have metalloregulatory function as defined above.

The chemistry of the iron-receptor site in Fur is complicated by our recent observation of a Zn(II) requirement for any DNA binding activity. Fur tightly binds one zinc ion per monomer in a cysteine rich environment. One zinc is, however, not sufficient for DNA binding. A second less tightly bound zinc or iron ion is required. Transcription assays are underway to test whether the Fe,Zn-Fur complex is the physiologically active repressor.

Multinuclear NMR studies of the ^{199}Hg-substituted MerR protein provides insights into the coordination chemistry of the trigonal metal-thiolate receptor site. The S=1/2 ^{199}Hg nuclide is shown to be an outstanding probe of metal environments in other metalloproteins including blue copper, zinc finger and zinc hydroylases. While the receptivity of is lower than ^{113}Cd, the ^{199}Hg chemical shift is much wider than ^{113}Cd and coupling constants to sidechain protons are larger. Our work to date indicates that ^{199}Hg NMR is thus a more sensitive probe of coordination environments in zinc, copper and mercury metalloproteins than ^{113}Cd.

Novel Synthetic Pathways to Reduced Iron-Sulfur Clusters

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The conversion of Fe-S clusters is normally associated with oxidation/reduction of iron and replacement of ligands. Starting from the easily accessible Fe_4S_4 core we tried to synthesize Fe-S clusters with a lower mean oxidation state of iron. These reduced Fe-S clusters should serve as precursors for the synthesis of model compounds for the active sites of metalloproteins.

According to the schemes below clusters with the cores $[Fe_6S_6]^{2+}$ (see also [1]) and $[Fe_8S_6]^{4+}$ [2] could be isolated by simple reactions

$$[Fe_4S_4I_4]^{2-} \xrightarrow{\quad PR_3 \quad} [Fe_6S_6I_2(PR_3)_4]$$

$$[Fe_4S_4(SR)_2L_2] \xrightarrow{\quad PR_3 \quad} [Fe_6S_6(SR)_2(PR_3)_4] \quad (L = SC(NMe_2)_2)$$

$$[Fe_4S_4I_4]^{2-} \xrightarrow{\quad [FeI_2(PR_3)_2] \quad} [Fe_8S_6I_8]^{4-}$$

$$[Fe_6S_6I_2(PR_3)_4] \xrightarrow{\quad [FeI_3(PR_3)]^- \quad} [Fe_8S_6I_8]^{4-}$$
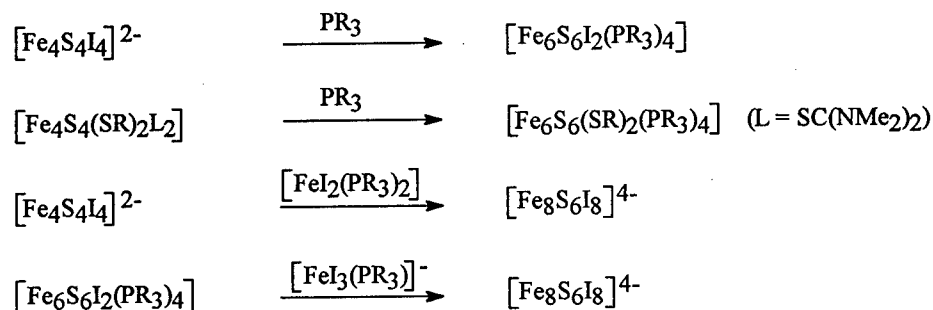
Another goal was the synthesis of mixed metal clusters. Starting again from Fe_4S_4 or Fe_6S_6 clusters we obtained compounds with the cores $[Fe_6Ni_2S_6]^{4+}$, $[Fe_4Ni_4S_6]^{4+}$ and $[Ru_2Fe_8S_6]^0$ [3], all of which have iron atoms in the oxidation state +II.

[1] B.S. Snyder and R.H. Holm, Inorg. Chem. **29**, 274 (1990) and lit. cited therein.

[2] U. Opitz and S. Pohl, Angew. Chem. Int. Ed. Engl. **32**, 863 (1993).

[3] S. Pohl, W. Barklage, W. Saak and U. Opitz, J. Chem. Soc. Chem. Commun. **1993**, 1252.

Ab Initio Molecular Dynamics Studies on
the Anticancer Drug Cisplatin Binding to DNA.

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We have investigated the interaction between the anticancer drug cis-diamminedichloroplatinum(II) (DPP) and DNA with ab initio molecular dynamics simulations[1]. In this approach the potential between atoms is calculated from first principles of quantum mechanics, within the framework of density functional theory, and no parametrization of the force field is needed. Starting from model built monofunctional adducts of DPP with d(pGpG), such the one represented in Fig. 1, we have described the formation of the final bifunctional adduct in terms of structural and energetic changes during the reaction path.

Figure 1. $Pt(NH_3)_2H_2O[d(pGpG(N7(1)))]$ adduct model built-structure. The H-bonds between the platinum ligands and the dinucleotide are indicated by dashed bonds. The adduct can react rapidly with the N7 of the second guanine to form the adduct $Pt(NH_3)_2[d(pGpG(N7(1),N7(2)))]^2$.
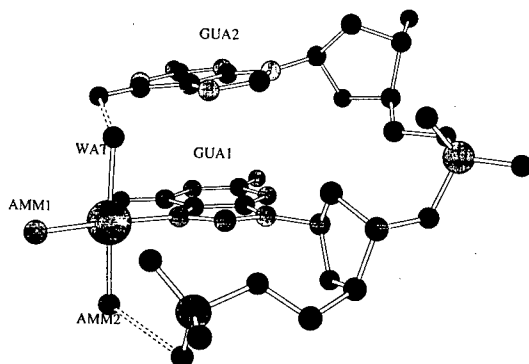
[1] R. Car. M. Parrinello, Phys. Rev. Lett., 1985, 55, 2471.
[2] D.P.Bancroft, C.A. Lepre, S.J. Lippard, J. Am. Chem. Soc., 1990, 112, 6860 and references therein.

Towards the 3D Structure of a Ferredoxin by Homonuclear NMR Studies

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The procedure for the structural determination of relatively small diamagnetic proteins in solution by NMR methods is well established[1]. However, the method has not been successfully applied to paramagnetic metalloproteins. The reasons for this are two-fold; firstly, the quality of the NMR spectra are often severely attenuated by the paramagnetism, resulting in non-assignment and/or lack of structural information around the paramagnetic centre. Secondly, reliable force-field parameters for metal ions coordinated to their proteins are generally not available.

Using conventional 2D homonuclear NMR experiments we have assigned ~ 50% of the backbone protons of the oxidised form of a $[Fe_4S_4]$ cluster ferredoxin from *Desulphovibrio africanus*. These protons are situated far away from the paramagnetic centre, and analysis of their NOE connectivities indicates that these regions mostly agree, in secondary structure at least, to the model predicted by homology studies[2]. It is clear, however, that both secondary and tertiary structural information around the $[Fe_4S_4]$ cluster is not amenable to conventional NMR techniques due to T_2 linebroadening which obscures NOE connectivities. We have therefore explored the possibilities of obtaining a tertiary fold where such information is unavailable. Using a variety of interproton distance constraints similar to those that are available from 2D NMR experiments, but derived from the X-ray structure of *Bacillus thermoproteolyticus* Ferredoxin it was possible to ascertain the likelihood of deriving a solution structure *ab initio* from NMR data. The calculated structures showed that reasonable agreement could be obtained if the effects of the paramagnetism was not far reaching (i.e. less than 6Å). In such cases the conserved geometry of the cluster and its ligands, in conjunction with the force field, is sufficient to determine the fold around the cluster. If the effects of the paramagnetism is greater, poorly resolved structures are obtained. In all cases, however, it is evident that the quality of the structure improves if distances involving protons close to the cluster can be obtained. We have therefore employed the method reported by Bertini et al.[3], to assign protons around the $[Fe_4S_4]$ and have succeeded in stereospecifically assigning peaks to the β-protons of the ligating cys residues.

References

1 Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids.* John Wiley and Sons, New York; Clore, G.M., Gronenborn, A.M. (1989) *CRC Crit. Rev. Biochem.,* 24, 479.
2 Fukuyama et al. (1988) *J. Mol. Biol.,* 199, 183.
3 Bertini, I., Capozzi, F., Ciurli, S., Luchinat, C., Messori, L., Piccioli, M. (1992) *J. Am. Chem. Soc.,* 114, 3332.

BINDING AND KINETIC STUDIES OF THE OXIDATION OF ALDOSES AND RARE SUGARS BY Cr^{VI}

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Cr^{VI} compounds are known to be toxic and carcinogenic; however, it has been proposed that Cr^{V} is the actual carcinogenic species. The tripeptide glutathione reduces Cr^{VI} to Cr^{III} but in the presence of sugars a Cr^{V} species is stabilized and the only requirement for complexation appears to be a cis arrangement of vicinal hydroxyl groups. In light of these observations Cr^{VI} and Cr^{V}-carbohydrate chemistry deserves more study[1].

Our project is concerned with the study of the interaction of aldoses[2], deoxyaldoses and amino compounds[3] with Cr^{VI} in acidic media as models for interpretation of interactions of macromolecules of biological importance, such as pectins and chitin, with Cr^{VI} in contamined waters. Kinetic results obtained for the chromic oxidation of D-mannose (M), 2-deoxy-D-mannose (2DM), 2-amino-2-deoxy-D-glucose (Glam) and N-acetyl-2-amino-2-deoxy-D-glucose (NAG) reveal that in every case sugars are oxidized to aldonic acids by slow electron transfer steps right after formation of a chromic ester. Cr^{VI} binds M and Glam through $OH(NH_2)$ on C_1 and C_2 to form SCr^{VI} species yielding the redox products. The lack of C_2-OH in 2DM compels Cr^{VI} to bind the anomeric OH only, and, consequently, a weaker ester is formed and rapidly oxidized to the products. Protection of C_2NH_2 in NAG could be thought to prevent NCr^{VI} binding and to afford kinetic results similar to those of 2DM. However, the greater kinetic formation constant suggests NCr^{VI} binding to be favored. Mechanisms, relative oxidation rates and stability of Cr^{V} esters at different pH, will be discussed.

1- D. Whitefield et al., Coor. Chem. Rev. *122*, **1993**, 171.

2- L. Sala, S. Signorella, M Rizzotto, Can. J. Chem. *70*, **1992**, 2046.

3- S. Signorella, C. Palopoli, V. Alba, L. Sala, Polyhedron *12*, **1992**, 2224

The catalytic centre of superoxide dismutase of

Propionibacterium shermanii

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The SOD of the Propionibacterium shermanii subspec.freudenreichii
is a polymeric enzyme with M = 23000 Dalton per monomeric unit.
The catalytic centre contains one Fe which can be replaced by Mn,
Cu and probably Zn without destroying the catalytic activity. The
SOD crystallized in the space group $C222_1$ with a=81.1A, b=85.5A,
c=108.8A. Crystals of Mn-SOD and Fe-SOD are isomorphous. EXAFS
experiments indicate a coordination of the iron with histidines.
The results are compatible with 4 nitrogens at a distance of 2.1A
and 2 oxygens at a distance of 1.9A. Mössbauer and EPR
investigations at the iron have been performed on azide ligated
SOD at pH 7.8 and on unligated SOD at pH 9.4, pH 7.8 and pH 6.4.
In all cases one obtains Fe(III) high spin with a strong rhombic
distortion. In the unligated SOD three conformations seem to be in
a pH dependent equilibrium. Each conformation shows a distribution
of conformational substates.

CONTRAST AGENTS IN MRI

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Contrast agents are used in Magnetic Resonance Imaging (MRI) in order to increase the contrast between signals originated in different tissues. The MRI signal is proportional to the proton spin density spatial distribution and to the proton longitudinal and transverse relaxation rates (T_1^{-1} and T_2^{-1}). We describe here the strategies to increase T_1^{-1} and T_2^{-1} of these protons by the use of contrast agents.

In order to have a strong dipole-dipole interaction between water protons and contrast agent, paramagnetic metals with a high number of unpaired electrons and long electron spin relaxation time have to be chosen (i.e. Mn^{2+}, Gd^{3+}). The toxicity of these metal ions has to be reduced by forming stable chelated complexes, i.e. with DTPA. Inevitably, this causes a decreasing of the relaxation rate, with respect to the metal ions, because of the lower number of sites available for water.

The correlation time τ_C, which modulates the coupling between water protons and unpaired electrons has to be increased as much as possible. Thus, the rotational correlation time τ_R is increased by binding of a metal ion chelate to a large molecule, like a protein, so that τ_C becomes equal to the electron relaxation time τ_S. Therefore, efforts have to be made in order to increase the electron relaxation time, which mainly depends on modulation in coupling between orbital angular momentum and spin magnetic moment[1]. In particular, we note that an increase in T_1^{-1} can occur because of the field dependence of the electron relaxation time.

Besides, if contact contribution is not negligible, the field dependence of electron relaxation time can provide a strong enhancement in T_2^{-1} at high frequency, independently from the τ_R value.

Simulations of Nuclear Magnetic Resonance profiles as function of magnetic field (NMRD) are calculated by using a new program[2] able to take into account both dipolar and contact contributions for any metal spin nucleus quantum number, any electron spin quantum number, any rhombicity of g-tensor, zero field splitting and hyperfine coupling of the electron spin system with the metal nucleus. These three effects can dramatically influence the NMRD profiles.

We suggest that a great increase in T_2^{-1} can also occur if the rotational correlation time is longer than the electron relaxation time, because Curie spin relaxation can become dominant.

[1] L. Banci, I. Bertini,. C. Luchinat, *Nuclear and Electron Relaxation*, VCH, Weinheim, Germany (1991).

[2] I. Bertini, O. Galas, C. Luchinat, G. Parigi, submitted.

Metal Complexes of Carbonic Anhydrase inhibitors. Synthesis and Characterization of Copper(II)-BOC-acetazolamide(B-H₂ats) complex. Crystal Structure of [Cu(B-ats)(NH₃)₂]₂ complex.

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The acetazolamide(H₂acm) as a therapeutically effective inhibitor of the carbonic anhydrase enzyme has encouraged the synthesis of structural derivatives with the aim of studying the effects of these changes on the inhibitory activity and pharmacological properties. Supuran[1] has shown that the metal complexes of sulfonamides are potent inhibitory agents of the carbonic anhydrase, this inhibitor capacity has been assigned to a double   action; the inhibition of the free sulfonamide in solution and the interaction of the metal with the histidin 64 at the active site of the enzyme.

In our attemps to prepare new acetazolamide derivatives (2), we have obtained the sulfonamide shown in the figure

$$(CH_3)_3O-\underset{\underset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-\underset{S}{\overset{N-N}{\diagup\diagdown}}-SO_2NH_2$$

B-H₂ats
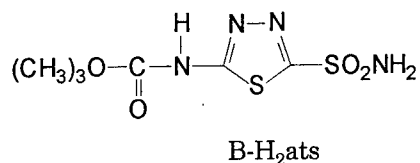
The new sulfonamide is a acetazolamide derivative. As consequence we have tried to obtain metal complexes in order to compare with those of acetazolamide and methazolamide. In the present investigation, the crystal structure of a dimer copper(II) complex of the B-H₂ats is reported. The coordination polyhedron around the copper is intermediate between square pyramid and trigonal bipyramid. The magnetic susceptibilities measured from 4.7K show a weak antiferromagnetic spin coupling.

1.- Supuran C.T., *Roum. Chem. Quater. Rev.*, **1**, 77 (1993).
2.- Pedregosa J.C., Alzuet G., Borrás J., Fustero S., García-Granda S. and Díaz M.R., *Acta Cryst.*, **C49**, 630 (1993)

Steric Influence on the Formation of Mixed-Valence 1-Methylthymine Compounds

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In recent years a great deal of interest has been devoted to studies on "platinum pyrimidine blues". These compounds possess antitumor activity, a feature possibly related to their cationic nature and the ability to interact with the anionic DNA. Several model complexes were structurally characterized and revealed that the compounds consisted of tetra- or octanuclear chain cations with formal average oxidation states of 2.25 - 2.50 [1-3].

Surprisingly, a dinuclear Pt(II)/Pt(III) species (Pt 2.50) has not been isolated as yet. Rather in all structurally established cases, Pt(2.50) is stabilized via dimer-of-dimer formation. Using dinuclear 1-methylthymine (1-MeT) complexes of type cis-$[a_2Pt(1-MeT)_2PtX_2]^{n+}$ (head-head) we reasoned that a possible way of avoiding the formation of a linear dimer-of-dimer structure would be to increase the steric bulk of the X ligand up to the point where intermolecular Pt⋯Pt contacts are prevented. On the other hand, if too big, the steric bulk between a and X ligand in the dinuclear cation might increase the Pt⋯Pt distance and therefore prevent further oxidation to a stable species. Applying X = CH_3NH_2 we could indeed observe in solution (CV, EPR, UV) the existence of a dinuclear Pt(2.50) compound. Surprisingly the use of $(CH_3)_2NH$ does not permit the observation of this compound
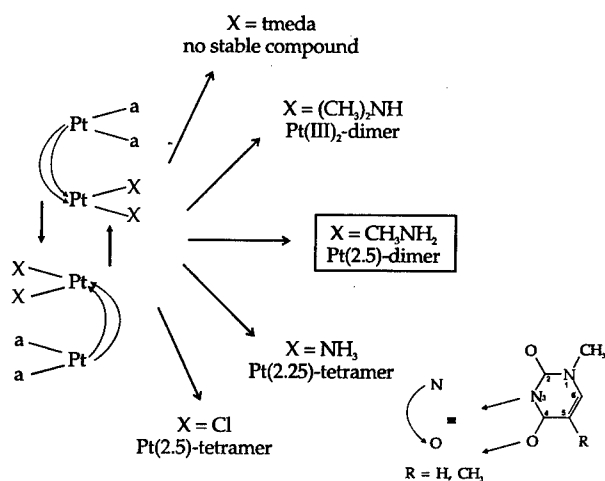
and only the oxidation to a Pt(III)-dimer is possible, while in the case of a tmeda-ligand even the Pt(III)$_2$-entity is not stable. Furthermore we will show that the removal of Cl in the compound cis-$(CH_3NH_2)_2PtL_2PtCl_2$ leads to interesting hydroxo-bridged dimer-of-dimer structures and we will discuss their oxidation behaviour and present the NMR data of multinuclear NMR studies (^{1}H, ^{195}Pt).

Lit.:

1. J. K. Barton, D. J. Szalda, M. N. Rabinowitz, J. V. Waszczak, S. J. Lippard, J. Am. Chem. Soc. **101**, 1434 (1979)..
2. K. Matsumoto, K. Sakai, K. Nishio, Y. Tokisue, R. Ito, T. Nishide, Y. Shichi, J. Am. Chem. Soc. **114**, 8110 (1992).
3. W. Micklitz, G. Müller, B. Huber, J. Riede, F. Rashwan, J. Heinze, B. Lippert, J. Am. Chem. Soc. **110**, 7084 (1985).

SPECTROSCOPIC STUDIES ON AN IRON-NITRIC OXIDE COMPLEX

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For the past decade, nitric oxide (NO) has been implicated in a number of diverse physiological and pathophysiological processes.[1] NO readily forms complexes with transition metal ions, including those regularly found in metalloproteins. In biochemical literature, interest has focused on its reactivity toward iron-sulfur centers in proteins, such as aconitase.[2]

It was first demonstrated in 1965 that reaction between iron salts and nitric oxide in the presence of anionic ligands gave rise to a four-coordinated complexes, of general stoichiometry $[Fe(NO)_2L_2]^{x+}$. This specie is characterized by EPR spectra with isotropic g-values in the range of 2.02 to 2.04. In the specific cases where L is a cystein residue a stoichiometry of $[Fe(NO)_2(SR)_2]^{1-}$ has been proposed.[3]

Here we report a detailed spectroscopic characterization of a complex between Fe, NO and cystein. Characteristic hyperfine parameters were determined from Mössbauer and EPR measurements. Structural significance of these parameters and functional implications of the complex in relation to NO reaction with Fe-S proteins are discussed.

1. Henry, Y., Ducrocq, C., Drapier, J.-C., Servent, D., Pellat, C., and Guissani, A. 1991. *Eur. Biophys. J.* **20**, 1-15.

2. Kennedy, M.C., and Beinert, H. 1994. *J. Inorg. Biochem.* In press

3. McDonald, C.C., Phillips, W.D., and Mower, H.F. 1965. *J. Am. Chem. Soc.* **87**, 3319-3326.

ISOLATION AND CHARACTERIZATION OF A HIGH MOLECULAR WEIGHT CYTOCHROME FROM THE SULFATE REDUCING BACTERIUM *DESULFOVIBRIO GIGAS*

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ABSTRACT

A high molecular weight c-type cytochrome was purified and characterized from *Desulfovibrio gigas*. The molecular weight was estimated to be 67 kDa by SDS/PAGE. The protein contains 16 hemes and its N-terminus is homologous to those of high molecular weight cytochromes c from *Desulfovibrio vulgaris* strains Hildenborough and Miyazaki. The purified hemoprotein shows c-type cytochrome absorption spectrum with $\varepsilon_{553}(\text{red}) = 368$ mM^{-1}cm^{-1}. A band at 640 nm, characteristic of high-spin hemes, is clearly detected. The EPR spectra show the presence of two high-spin heme species, plus several non-equivalent low-spin hemes. The heme reduction potentials, at pH 7.6, range from -50 mV to -315 mV. In contrast to what has been described for *D.vulgaris* Hmc, the protein isolated from *D.gigas* accepts directly electrons from hydrogenase and further reduces other redox proteins.

IRON UPTAKE - BINDING TO AND PENETRATION THROUGH THE INTESTINAL BRUSH BORDER MEMBRANE

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Due to the high binding capacity of the brush border membrane (BBM) for metal ions, it is very important to distinguish between binding to and penetration through the membrane, when studying iron uptake. Using BBM-vesicles, there are four possible compartments, where the substrate could be located, namely

      a) outer membrane surface

      b) inner membrane surface

      c) membrane interior

      d) aqueous phase within the vesicles.

A localisation strategy by running Sephadex colums has been developed. This includes the exchange of iron bound to the outer membrane surface with a tracer free medium or a separation of the vesicles from the medium using a Sephadex column. To measure the iron bound strongly to the membrane, an EDTA incubation is applied. After lysis of the vesicles with Triton-X100, the amount of iron within the vesicles becomes detectable. Finally, the separation of iron bound within the membrane and on the inner surface can be achieved by means of a simultaneous EDTA incubation during the lysis and a subsequent run through a Sephadex column.

The amount of iron present in each vesicle compartment was determined after an incubation with a medium containing 200 µM iron chelated with nitrilotriacetate (NTA) in a ratio 1:2. About one third of the iron was bound on each side of the brush border membrane, one quarter was located within the membrane, and the amount of iron in the aqueous phase within the vesicles (ca. 8%) corresponded approximatively to the equilibrium with the medium iron concentration.

The Iron-Quinone Complex of Photosystem II

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Photoexcitation of the Photosystem II (PSII) reaction center drives single electron transfer from the primary electron donor, P680, a probable dimer of chlorophyll a, to a terminal e^- acceptor, an array of a plastoquinone molecule, Q_A, an Fe^{2+}-histidine complex, and a secondary plastoquinone, Q_B. Q_A is a single electron acceptor while Q_B accepts two electrons and two protons. The non-heme Fe^{2+} is located in the middle of the two quinones and interacts magnetically with either of the semiquinone radical anions, as this is manifested by characteristic EPR signals in the $g=1.8-1.9$ region. The iron does not appear to participate directly in electron transfer, but the electron transfer rate between the two quinones is strongly decreased in the absence of HCO_3^-/CO_2, which is a labile ligand to the iron. A number of properties of the iron are interesting in this respect: The iron is redox active and in the oxidized form is characterized by EPR signals at $g=8$ and 5.6. Ligand exchange studies suggest that NO, CN (Sanakis et al, these proceedings) and a number of carboxylate anions (Deligiannakis et al, these proceed.) bind readily to the iron in competition with HCO_3^-/CO_2. The Mossbauer and EPR properties of the iron bear striking similarities with lipoxygenase and with a number of dioxygenases, and the binding of bicarbonate and of a number of carboxylate anions suggests similarities with transferrin. The physiological role of the iron at the present level of understanding is, however, unique. It appears that the iron controls indirectly the electron transfer rate and therefore the oxygen evolution depending on the intracellular CO_2 levels.

^{1}H NMR Spectroscopy and Coordination Structure of Cobalt(II) Cys112Asp Azurin

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^{1}H NMR spectra of Co(II) substituted Cys112Asp azurin from *Pseudomonas aeruginosa* have been analyzed and compared with those of the Co(II) wild-type protein. Hyperfine shifted signals (including Asp 112 β-CH$_2$ signals in the mutant as well as previously unobserved Cys 112 β-CH$_2$ signals in WT) from all the metal-coordinated residues have been detected and unambiguously assigned. Notably, the spectra indicate that very little if any unpaired spin density is located on the Met 121 protons in the Cys112Asp protein. A computer-generated model of the mutant Co(II) structure consistent with electronic absorption as well as the NMR data includes a Gly-45 carbonyl, His 46, an unusually coordinated Asp 112 and His 117 in the ligation sphere.

The Bond Valence Sum Approach to the Determination of the Structure at Metal Centres in Biological Systems.

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Equation (1) relates the oxidation state (V_i) of a metal ion (i) to a sum of the bond valences(s), calculated for each metal ligand bond using equation (2); where r is the bond length, B is currently assigned a value of 0.37 and r_0 is a constant for a particular atom pair.

$$V_i = \Sigma_j \, s_{ij} \qquad (1) \qquad\qquad s = \exp((r_0 - r)/B) \qquad (2)$$

Brown and Altermatt(1) derived values of r_0 for many cation-anion pairs from structural information available in the Inorganic Crystal Structure Database for infinite 3D-solids. Thorp(2) has applied equations (1) and (2) to molecular compounds. He derived values of r_0 for a few transition metal-ligand atom pairs from the bond lengths in suitable model complexes and showed the oxidation states of the metals in several metalloenzymes calculated from equation (1) generally agreed with the accepted assignment.

We are investigating another possible application of equations (1) and (2). If reasonably reliable values of r_0 can be calculated from the structural data available for coordination complexes, equations (1) and (2) could be used to obtain coordination numbers of metals in metalloenzymes, in cases where the oxidation state and the metal-ligand distances have been measured. This approach is particularly relevant to X-ray absorption spectroscopy which provides information concerning the oxidation state (edge position) and the dimensions of the inner coordination sphere (EXAFS), but rarely provides coordination numbers with a precision better than ± 1.

This approach has been tested and, for example, the results are in agreement with earlier predictions that the Cu^I-N distance of 1.94Å in reduced bovine superoxide dismutase (3) and the Cu^I-S distance of 2.25Å in Cu/Zn- metallothionein(4) are both indicative of three-fold coordination. Also, the vanadium environment in both native and reduced bromoperoxidase appears to be five coordinate(5).

1. I.D. Brown and D. Altermatt, *Acta Cryst.*, **B41**, 244 (1985)
2. H.H. Thorp, *Inorg, Chem.*, **31** 1585 (1992)
3. N.J. Blackburn, S.S. Hasnain, N. Binstead, G.P. Diakun, C.D. Garner, and P.F. Knowles, *Biochem. J.*, **219**, 985 (1984)
4. I.L. Abrahams, C.D. Garner, I. Bremner, G.P. Diakun and S.S. Hasnain, *Biochem.J.*, **236**, 585 (1986)
5. C.J. Carrano, M.Mahon, S.Holmes, R de la Rosa, A.Butler, J.M Charnock and C.D. Garner, *Inorg. Chem* , **33**, 646,(1994)

EPR SPECTROSCOPIC STUDIES ON THE P CLUSTERS OF MOLYBDENUM, VANADIUM AND ALTERNATIVE NITROGENASE SYSTEMS.

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Three distinct classes of nitrogenases are now known. Systems containing a molybdenum or vanadium atom in their cofactor centers are well-characterized. The third (alternative) nitrogenase system is currently thought to have an Fe-only cofactor which contains neither Mo nor V [1]. Comparison of primary sequences, aided by the X-ray crystallographic structure of two MoFe proteins, shows that it is likely that cofactors in the V and alternative systems are also bound by histidine and cysteine. Conservation of the six cysteine residues in the *vnfDK* and *anfDK* genes, which in Mo-containing systems are ligands to P clusters, strongly suggests their presence in the vanadium and alternative nitrogenases. We have now substantiated this hypothesis by the detection of two-electron oxidized P clusters,
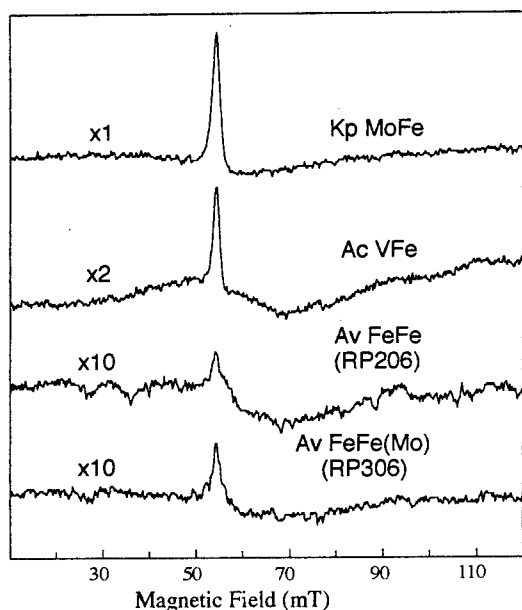
FIGURE 1. Comparison of the parallel-mode EPR spectra of the oxidized P clusters in component 1 of various nitrogenase systems. (200 mW, 16 K).

which exhibit a characteristic g = 12 integer spin EPR signal (see [2] and Figure 1). (E_m = -145 mV for Ac1^V and -185 mV for Av1^{FeFe} compared with $\approx$ - 300 mV for Av1 and Kp1). The pH dependence of the two-electron oxidation of the P clusters of Kp1 indicates a coupling of proton release and oxidation. The disproportionation of the one-electron oxidized P cluster species is highly pH dependent.

1. B.E. Smith and R.R. Eady, *Eur. J. Biochem.*, **205**, 1 (1992)
2. A.J. Pierik, H. Wassink, H. Haaker and W.R. Hagen, *Eur. J. Biochem.*, **212**, 51 (1993)

NEURONAL VOLTAGE-DEPENDENT CA^{2+} CHANNELS

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Voltage-dependent Ca^{2+} channels mediate Ca^{2+} influx into neurons that is crucial for many processes in the brain including dendritic spiking, neurotransmitter release, synaptic plasticity, gene expression and cell death. Under physiological conditions Ca^{2+} channels show selectivity for Ca^{2+} over more plentiful ions such as Na^{+} or K^{+} by virtue of their high affinity binding of Ca^{2+} within the pore. Ca^{2+} channels are multisubunit structures composed of the voltage-sensitive pore-forming α_1 subunits, the regulatory ß and α_2-δ subunits and perhaps additional subunits. Considering the different genes for α_1 and ß subunits, the different splice variants for each subunit, and the many possible subunit combinations, a functional diversity of brain Ca^{2+} channels much wider than the established pharmacological diversity can be expected in native neuronal membranes. With the aim of characterizing the functional and pharmacological properties of all the different voltage-dependent Ca^{2+} channels expressed in a given type of C.N.S. neuron we obtained single Ca^{2+} channel cell-attached patch-clamp recordings from rat cerebellar granule cells in primary culture. Our results show that these neurons coexpress at least five different types of Ca^{2+} channels: two functionally different dihydropyridine-sensitive L-type Ca^{2+} channels and three functionally different Ca^{2+} channels resistant to both dihydropyridines and ω-conotoxin-GVIA. Four of these Ca^{2+} channels had not been previously described in cellular membranes.

REDOX-LINKED SPIN STATE CHANGE IN *Paracoccus denitrificans* CYTOCHROME*c* PEROXIDASE

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A diheme cytochrome *c* peroxidase from *Paracoccus denitrificans (Pa.d.)* has been characterized spectroscopically by UV/Visible, NMR, EPR and Mössbauer [1-3]. This enzyme, responsible for the remove of hydrogen peroxide from cells, catalyzes its reduction to water.

Pa.d. peroxidase (40 kDa) has some similarities to the one isolated from *Pseudomonas aeruginosa.* Both enzymes have two *c*-type hemes with very different oxidation-reduction potentials. This allows the designation of the hemes as high-potential (HP) and low-potential (LP) hemes. The HP heme, with a Met-His axial coordination, constitute the <u>electron transfer domain</u>; the LP heme, with a possible His-His or His-Lys coordination, constitute the <u>peroxidatic domain</u>.

The *Pa.* peroxidase bounds calcium ions near the hemes, which are required for enzyme activation. This observation makes an important difference between the *Pa.* and the *Ps.* enzymes.

Cytochrome *c* peroxidase, as isolated, is in a catalitically inactive oxidized form (both hemes are ferric). The HP heme is high-spin and the LP heme is low-spin. The reduction of the HP heme with ascorbate (half-reduced enzyme) changes its spin state from high to low-spin. This form of the enzyme is still inactive; only after the conversion of the LP heme into a high-spin state, promoted by the addition of calcium ions, the enzyme becomes active and competent for hydrogen peroxide reduction.

These processes of reduction and spin state change can be separated stepwise after treating the enzyme with EDTA, in order to remove the bound calcium ions.

1. Gilmour, R., Goodhew, C.F., Pettigrew, G.W., Prazeres, S., Moura, I. and Moura, J.J.G. (1993) *Biochem. J.* **2941**, 745-752.
2. Prazeres, S., Moura, J.J.G., Moura, I., Gilmour, R., Goodhew, C.F. and Pettigrew, G.W. (1993) *Magn. Reson. Chem.* **31**, S68-S72.
3. Gilmour, R., Goodhew, C.F., Pettigrew, G.W., Prazeres, S., Moura, J.J.G. and Moura, I. (1994) *Biochem. J.* in press.

ANTIOXIDANT ACTIVITY OF COPPER(II) COMPLEXES

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Molecular Oxygen is essential for living organism. However, during its reduction to water, toxic intermediates as superoxide radicals, hydrogen peroxide and hydroxyl radicals are formed, according to the following scheme;

$$O_2 \xrightarrow{e^-} O_2^{-} \xrightarrow{e,H^+} H_2O_2 \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e,H^+} H_2O$$
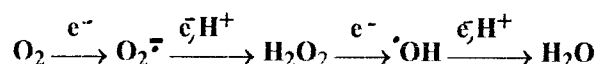
SOD (superoxide dismutase) is the metal-enzyme that catalyzes the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide, the last being converted into water plus oxygen by catalase. The implication of oxygen radicals in numerous pathologies has induced many researchers to study the use of SOD as therapeutic agent. Owing to the limits surrounding the use of this enzyme as a drug, a certain number of low molecuale weight compounds which mimic SOD have been synthesized. Manganese(III), Iron(III) or Copper(II) complexed with ligands, either synthetic or of biological origin, have been tested and used for their capacity to act as efficient catalyst in the dismutation of the superoxide radical.

Recently, we have thermodinamically and spectroscopically characterized copper(II) complexes with newly synthetized ligands, tailored to optimize the superoxide radicals scavenger ability. The relevance of speciation studies will be also discussed togheter with proposed structure-activity relationship.

Synthesis and Crystal Structure of Mononuclear and Dinuclear Iron(III) Complexes with a Terminal Coordination of Benzimidazole and Phenolic Residues of a New Polydentate Ligands: Models for the Active Site of Purple Acid Phosphatases

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Purple acid phosphatases (PAP) are a group of non-heme dinuclear iron-oxo proteins catalyzing the hydrolysis of activated phosphoric acid esters under acidic conditions. A proposed structure of the phosphate complex of the oxidized diiron center has two iron(III) atoms bridged by one carboxylate, one oxo and one phosphato group. Iron centers are unsymmetrically coordinated with oxygen (tyrosine, aspartate or glutamate) and nitrogen (histidine) based amino acid residues. Because of the lack dinuclear iron model complexes with unsymmetrical coordination by histidine- and tyrosine- like ligands in the literature, we have developed a synthetic route for the preparation of a potentially dinucleating unsymmetrical ligand, 2-[bis(2-benzimidazolylmethyl) amino]methyl-4-methyl-6-[(2-methylbenzimidazol)-(2-methoxy-phenylmethyl)amino]methyl-phenol (**LMeO**), which should provide (after deprotection of the phenoxy group) the desired unsymmetrical coordination. Two dinuclear iron(III) complexes, with di-μ-hydroxo (**1**) and phosphato (**2**) bridging ligands, respectively, show an unexpected coordination of two ligands per dinuclear center, as revealed by single crystal analysis (Fig. 1). When the HCl salt of LMeO was used, the mononuclear complex (**3**) was isolated which can possibly serve as a precursor for the preparation of a mixed metal dinuclear complexes with a different coordination of the two metal centers. An attempt to synthesize a phosphato- bridged dinuclear Fe(III) complex using N-(2-Benzimidazolylmethyl)-N-(2-hydroxyphenylmethyl) amine leads to the formation of a mononuclear complex (**4**) with diphenylphosphate as an uncoordinated anion, indicating a high stability of this coordination geometry, which is reminiscent of several non-heme proteins with mononuclear iron active sites.

(1) (2)
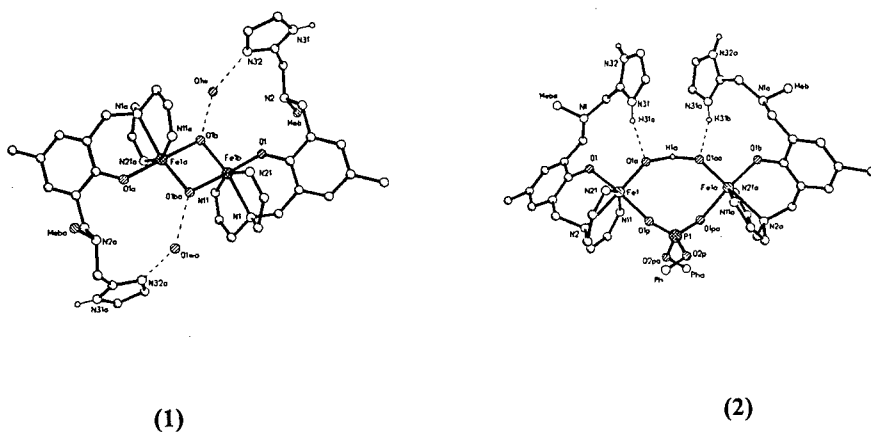
Fig. 1. Simplified stereoplots of the dinuclear Fe(III) complexes (**1**) and (**2**), showing coordination spheres and the role of the hydrogen bonding in both structures. Most benzimidazole atoms are not shown for clarity. MeB: 2-methoxybenzyl; Ph: phenyl

Synthetic Coordination Chemistry Inspired by Metalloprotein Active Sites

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The active site in metalloproteins and especially the coordination chemistry of the metal ions in metalloproteins and metalloenzymes has fascinated chemists for the last few decades. Following the successful research to mimic the active sites of vitamin B-12 and myoglobin, researchers have been increasingly inspired by the idea to mimic the structure, the spectroscopy and possibly the activity of a metalloprotein.

Three major approaches have been followed worldwide:

1. Speculative models, to mimic a site of a not yet structurally characterized metalloprotein; i.e. photosystem-II, and – till recently – nitrogenase.

2. Corroborative models, to study small variations in structure and conformation of a site mimicking a (fully) characterized metalloprotein; examples are models for myoglobin, blue copper proteins, cytochromes.

3. "Applicative" or "functional" models, to study the reactivity (catalysis, mechanism) of species resembling the (known) site of a metalloprotein; examples to be mentioned are the reactions with P-450 models, models for methane monooxygenase.

Mimics from the last category are currently considered as a part of "bioinorganic catalysis", a branch of catalysis in which the catalyst and the process originate from the biological sciences and which is dealing with enzymes. The research programs in inorganic chemistry, biochemistry and catalysis have successfully met and the results are exciting. In fact, many ideas in present-day catalysis research have had an inspiration from some enzymatic reaction or from an enzyme active-site structure (see ref. 1 and 2).

The lecture will focus on synthetic metal-containing molecules resembling the active site of metalloproteins. Strategies to design and synthesize analogs of the enzymes will be explained in the context of supramolecular chemistry. An important aspect for research in the future will be increasing attention for new, clean and selective catalytic processes, yielding useful, harmless and biodegradable products for the world society. Some examples based on our own work will be discussed in this round-table session.

1. J. Reedijk, *"Bioinorganic Catalysis"*, Marcel Dekker, New York, 1993.
2. S.J. Lippard et al., *A Special Issue on Bioinorganic Chemistry*. Science Magazine, **261**, (1993), pp. 699 - 730.

Dinitrogenvanadates(-I): Synthesis, Reactions, and the Conditions for and Limits of Their Existence

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The reduction of $VCl_3(thf)_3$ with sodium or lithium, M, in the presence of various mono- to tetradentate phosphines, p_m, yields $M[V(N_2)p_5]$ and *cis/trans*-$M[V(N_2)_2p_4]$, with an optimum of stability for $p_4 = 2$ dmpe. An intermediate V^I/V^{II} complex of composition $V_2p_6Cl_3$ has been identified. The N_2 complexes have been characterized by, *inter alia*, ^{51}V NMR spectroscopy (see Fig. 1 for *cis*-$Na[V(N_2)_2(dmpe)_2]$), ^7Li NMR (see Fig. 2 for *cis/trans*-$Li[V(N_2)_2(dmpe)_2]$) and X-ray crystallography (see Fig. 3 for *trans*-$[Na(thf)][V(N_2)_2(dppe)_2]$). The molecular structure in the solid state and the temperature-dependent ^7Li NMR spectra in solution reveal close contact-ion pair interaction of the kind $V-N\equiv N\cdots M$, which is essential for the stability of the compounds. Crown ethers initiate immediate decomposition. In the presence of CO or CN*t*Bu, the N_2 ligands are exchanged. HBr converts 25% of the N_2 in $[V(N_2)_2p_4]^-$ to NH_4^+ (plus a small amount of $N_2H_5^+$). The relevance of this observation is discussed in the context of functional models of vanadium-nitrogenase.
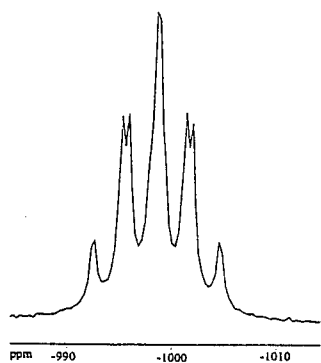
Fig. 1. ^{51}V NMR of

cis-$[V(N_2)_2(dmpe)_2]^-$
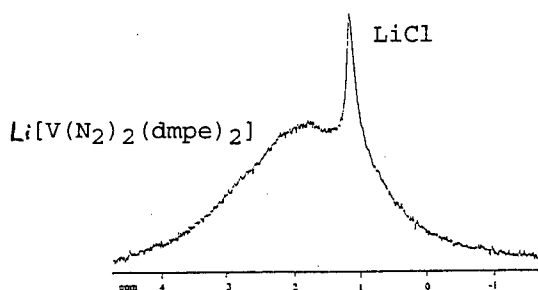
Fig. 2. ^7Li NMR at 203 K in THF
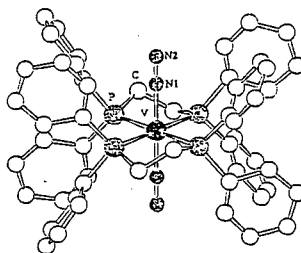
Fig. 3. Molecular structure of $[V(N_2)_2(dppe)_2]^-$

D. Rehder, C. Woitha, W. Priebsch, H. Gailus, *Chem. Commun.* 1992, 364.

H. Gailus, C. Woitha, D. Rehder, *Dalton Trans.*, submitted

Preliminary ¹H NMR Investigations of Manganese superoxide dismutase from Escherichia Coli

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We report the ¹H NMR spectrum of the enzyme Manganese Superoxide Dismutase from *E.coli*. This is the first NMR spectrum of a Mn(III) enzyme, notwithstanding with the severe limitations caused by the short relaxation times and the low concentration of the protein.

Some broad signals are observed, both downfield and upfield shifted, in the region +30/-30 ppm, with T_1 values of a few milliseconds. By dissolving the protein in D_2O, some of the upfield signal disappear, thus suggesting that at least some of the NH histidine protons are shifted upfield. The above results is consistent with what found in some models complexes of the enzyme.

As apoSOD can be prepared and reconstituted with different metal cofactors (however without recovery of the catalytic activity despite their structural likeness), we have introduced a better NMR probe, cobalt(II), in the active site of Mn-SOD form *E.coli*. The ¹H NMR spectrum of this Co-SOD derivative shows several hyperfine downfield shifted signals, arising from metal coordinated residues. Consistently with what expected for a five coordinated Co(II) chromophore, several resonances are observed, out from the diamagnetic envelope, in the region 20/-10 ppm. They should be ascribed to residues in the proximity of the metal center but not directly coordinated to the Co(II) ion. Preliminary NOE's have been performed to assign the resonances of metal ligands.

EPR, ESEEM AND ENDOR STUDY OF 2FE2S-CENTERS.
A COMPARISON OF RIESKE- AND FERREDOXIN-TYPE CLUSTERS.

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2Fe2S centres are usually divided into two distinct groups on the basis of their EPR spectra and redox midpoint potentials (E_m). The ferredoxin-type clusters are characterized by an average g-value (g_{av}) of 1.96 and a negative midpoint potential around -400 mV, whereas the Rieske centres show a pronounced g-factor anisotropy with g_{av}=1.91 and redox midpoint potentials around +300 mV. The high E_m of the Rieske centres was supposed to arise from a different coordination of the cluster compared to ferredoxin-type centres.

However, a detailed comparison of a variety of Rieske-centres shows that their redox potentials encompass the full range of 500 mV: the Rieske centres of the cytochrome bc complexes titrate around +300 mV and +130 mV depending on the type of quinone functioning as electron donor in the membrane (1,2), whereas the so-called Rieske-type centres of the bacterial dioxygenases have an midpoint potential around -100 mV and come close to the ferredoxin-type clusters with an E_m up to -170 mV. We present ESEEM and ENDOR spectra showing that the general model of the Rieske centres has to be modified. Other factors than the coordination of the cluster (e.g. the hydrogen bonding network) has to be taken into account to explain the differences between the individual Rieske centres.

1. U.Liebl, S.Pezennec, A.Riedel, E.Kellner and W.Nitschke, *Journal of Biological Chemistry* 267, 14068 (1992)
2. A.Riedel, E.Kellner, D.Grodzitzki, U.Liebl, G.Hauska, A.Müller, A.W.Rutherford and W.Nitschke, *Biochim. Biophys. Acta* 1183, 263 (1993)

Structure-activity relationships in copper-zinc superoxide dismutase.

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The three dimensional structure of one of the two Cu, Zn superoxide dismutase isoenzymes from the amphibian Xenopus laevis (X SOD B) has been defined at 1,5 A. Since this is the highest resolution available for this class of enzymes and recombinant mutants of the protein can be engineered, a project has been started in order to detail the substrate - active site interactions in Cu, Zn superoxide dismutase. The 3D structure of Cyanide and azide adducts of the enzyme has been studied by X ray cristallography as these anions are established to mimic the binding of the superoxide anion to the catalytic copper center. The data obtained confirm the distortion of the metal geometry and the key role of Arg 141 in the anion binding process, already pointed out by EPR and NMR spectroscopy. Data on enzyme activity have been collected by pulse radiolysis on a set of mutants involving electrostatically charged residues around the active site.

The results obtained can be summarized as follows, with respect to those already available for the human enzyme and brownian dynamics calculations carried out on the bovine enzyme: a) the same residue can have a different functional weight in different Cu Zn superoxide dismutase depending on its distance from the copper and the network of interacting charges surronding it.; b) mutants that are more active than the wild type enzyme at physiological ionis strenght can be engineered only by multiple mutation, because only combined neutralization of opposite charges can abolish the stronger salt dependence of single electrostatic mutants.

Prostaglandin H Synthase
Metal Substitution, Structure and Catalytic Mechanism

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Prostaglandin H synthase (PGHS, PG endoperoxide synthase, EC 1.14.99.1) is a key enzyme of prostaglandin biosynthesis and catalyses the conversion of arachidonic acid to prostaglandin H_2 in a two-step reaction. In the first step, the cyclooxygenase reaction, two molecules of O_2 are incorporated into the substrate, yielding the hydroperoxide PGG_2. In the second step, the peroxidase reaction, PGG_2 is reduced to PGH_2. Both steps are catalysed by the same enzyme, a protein of 72 kDa with one ferric heme as the prosthetic group. Its X-ray crystal structure determination was completed to a resolution of 3.5 Å recently (1) and revealed His-388 as the axial ligand of the iron.

The catalytic role of the heme is the direct participation in the peroxidase reaction analogous to other heme peroxidases, and also the initiation of the cyclooxygenase reaction, tentatively by the oxidation of a tyrosyl residue to a tyrosyl radical. This tyrosyl radical was suggested to abstract a hydrogen from the substrate as the initial step of the cyclooxygenase reaction (2). Metal substitution experiments show that only redox-active metals can reconstitute either enzymic activity. Zn-PGHS and Ga-PGHS are completely inactive. This reaction model is corroborated by the structure of the enzyme which reveals Tyr-385 at a distance of about 10 Å from the heme and at a position suitable for the proposed H-abstraction from C-13 of arachidonic acid.

1. D. Picot, P. J. Loll and R. M. Garavito (1994) Nature 367, 243-249.
2. H. H. Ruf, U. Raab-Brill and C. Blau (1993) Biochem. Soc. Trans. 21, 739-744.

CLINICAL EXPERIENCES IN THE USE OF CASIOPEINAS®(1) FOR THE TREATMENT OF SPONTANEOUS CANCER IN VETERINARY MEDICINE.

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Due to the potential benefits of using the new copper (II) coordination compounds (2, 4), casiopeínas, (I, II, III) various cases of spontaneous cancer in dogs and cats were treated either along or combined with surgery.

The doses rates and intervals were established by extrapolating data from toxicological studies in rats, mice and dogs. The selection of each casiopeína was based upon *in vivo* murine models of tumor cells lines transplantation (3).

In all, 11 dogs and 4 cats have been treated with the various casiopeínas: of which 4 osteosarcomas (casiopeína I), 1 leiomyiosarcoma (casiopeína I), 2 transmissible venereal tumors (casiopeína II), 4 mammalian carcinoma (casiopeína I). The 4 cats were treated with casiopeina III for various forms of feline viral leukemia (FVL). The success rate in dogs can only be assessed in terms of increments in their life expectancies (LE). From this point of view it can be reasonably stated that when the cancer was in early stages and surgery was carried out, casiopeínas can increase LE. In cats positively diagnose though ELISA and suffering the active form of the FVL, casiopeina III showed an outstanding ability to reduce leukocytes blood count and increase their LE. Furthermore 2 out of the 4 cats are still alive after six months from diagnoses. This preliminary apparent successful treatments main encourage other clinicians

1. Ruiz A.L, U.S.A Patent, april 21 (1992) No. 5,107,005

2. Ruiz-Ramírez L. *et al*. Stability of Ternary Copper and Nickel Complexes with ....J. Inorg., Biochem. 47 (1992), 121

3.- Ruiz-Ramírez L., Gracia-Mora I, *et al*.: Cytostatic, mutagenic, antineoplastic activities and preliminar toxicity of coppor (II) new drugs: Casiopeínas I, II, III. The Antitumor Activity of Several Transition Metal Complexes. J. of Inorg. Biochem.; (1993) 51: 1-2.

4.- Ruiz-Ramírez L. *et al*. Mixed Chelate Complexes II. Structures of....Acta Crys. 1993, C49, 890-893.

STUDY OF THE INTERACTION OF CASIOPEINAS®(1) WITH DNA AND BASES-CASIOPEINAS ADDUCTS.

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Gracia-Mora Isabel*, Ferrer-Sueta Gerardo*, Bravo-Gómez María Elena, Garcia-Carrancá Alejandro♦

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In view of the potential benefits of using the new copper coordination compounds (2, 3), casiopeinas, (I, II, III) as antineoplastic drugs (1,2), we have started a study of the possible mechanism of action of this mixed chelate Cu(II) complexes, Casiopeinas I, II and III. The casiopeinas are Cu(II) coordination compounds analogs among them and are classified in four sub- families , I, II III and IV, depending on the substituents on the general skeleton of the molecule [CuAB] NO_3 (4).

It has been shown that Casiopeina I produces small mutagenic changes upon studies with *Drosophila melanogaster* (2), such an evidence let us propose that the way of action of this drugs is through a DNA interaction.

For the above reasons we have perform a series of experiments in order to obtain information about the mechanism of action of this drugs. The compounds studied are from the casiopeinas I, II and III.

The studies are, 1- electrophoresis of DNA (4000 bp) (linear and super coiled), in agarose gel at different times (0, 10, 15, ,30 min.) at room temperature and 37°, and four concentration of drug. From this experiments we conclude that there is an increase of effect as a function of the concentration. Compounds of casiopeina I interact stronger with lineal DNA than with super coiled DNA; whereas the casiopeina II family compounds present an stronger interaction with super coiled DNA instead of linear DNA. For casiopeinas III the interaction is weaker in general.

The second method is a method in solution mixing the drug and the DNA and increasing the concentration , following the effect spectroscopically. Scachard pots were obtained. And a relation two to one was obtained.

Finally the reactivity of several casiopeinas I ,II and III with bases (adenine, timine guanine, tirosine and the nucleoside AMP was carried out. The analysis of the product obtained indicated that timine reacts less than the other bases. Results of this compounds are presented

1. Ruiz A.L., U.S.A Patent, april 21 (1992) No. 5,107,005

2. Ruiz-Ramírez L.. *et al.* Stability of Ternary Copper and Nickel Complexes with ....J. Inorg . Biochem. 47 (1992), 121

3.- Ruiz-Ramírez L., Gracia-Mora I, *et al.*: Cytostatic, mutagenic, antineoplastic activities and preliminar toxicity of copper (II) new drugs: Casiopeinas I, II, III. The Antitumor Activity of Several Transition Metal Complexes. J. of Inorg. Biochem.; (1993) 51: 1-2.

4.- Ruiz-Ramírez L. *et al* Mixed Chelate Complexes II. Structures of ...Acta Cryst. 1993, C49, 890-893.

THE INTERACTION OF APO-HEMOCYANIN WITH Cu(II) IONS

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The metal depleted active site of hemocyanin (Hc) behaves as a
polyimidazole cluster capable to bind a variety of metal ions. Thus, the
coordination of Cu(I) ions regenerates the dinuclear copper site of the
native oxygen binding protein. Derivatives of Hc containing Co(II) ions
(mononuclear or dinuclear) or Cd(II) ions (mononuclear) specifically in the
active site have been already prepared. Incubation of apo-Hc with Cu(II)
ions [0.5 mM $Cu(NO_3)_2$] generates a derivative binding a total number of
six Cu(II) ions per protein subunit. By EDTA treatment, the metal-to-
protein stoichiometry is reduced to 1 metal ion per subunit. The intrinsic
fluorescence of the apo-protein is quenched upon Cu(II) binding: the
quantum yield of the EDTA stable mononuclear Cu(II) containing derivative
is approx. 60% reduced. These results strongly suggest that the metal
binding occurs in the Cu_A position in the active site, as defined by X-ray
crystallography. The other, EDTA sensitive, Cu(II) ions are bound to
"peripheral sites". The reaction does not follow an apparent first order
kinetics, rather it can be described by a model in which dimers of subunits
allosterically interact during metal binding. Note worthy, incubation of apo-
Hc in a medium containing Cu(II), as indicated above, but with the addition
of azide (70 mM) results in the full regeneration of the native, oxygen
binding form demonstrating that a reduction of copper occurs. This reaction
can be described as a sequential model for the binding of copper to the two
metal binding positions in the active site (Cu_A and Cu_B).

CN⁻ and NO binding in Photosystem II.

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Cyanide is known to bind at the non-heme iron of the iron-quinone complex of the acceptor side of Photosystem II [Koulougliotis, D., Kostopoulos, T., Petrouleas, V., & Diner, B.A., (1993) Biochim. Biophys. Acta 1141, (275-282)]. Here we extend the study of the cyanide effects on the iron-quinone complex by EPR and Mossbauer spectroscopies.

We conclude that CN⁻ binds at the non heme iron in a two step mechanism. In the first step (pH 6.5) 1 or 2 CN⁻ bind at the non-heme iron. It is shown that although iron retains its electronic state ($Fe^{2+}(S=2)$) a change in the crystal field parameters occur. In the second step (pH=8.0), the binding of a 3rd CN⁻ results to the conversion $Fe^{2+}(S=2)$ to $Fe^{2+}(S=0)$.

Nitric oxide (NO) is known to bind reversibly and in competition with HCO_3^- at the non heme iron of the iron quinone complex [Petrouleas V. and Diner, B.A., 1990, Biochim. Biophys. Acta 1015, 131-140]. Here we report on an additional effect of NO on PS II. Illumination of NO treated PSII membranes induces an EPR signal with characteristics typical of a nitroxide radical (NR). The light induced NR EPR signal requires the presence of specific components of the donor side of PS II while its evolution parallels the Mn release.

We conclude that a "hydroxylamine" adduct is formed during NO treatment of PS II membranes. This species is EPR silent in the dark. Illumination leads to its oxidation and the NR EPR signal is formed. The chemical structure of the species responsible for the NR signal and the pathway of electron donation are under investigation.

Quaternary structures of molluscan and arthropod hemocyanins from SAXS and Electron Microscopy.

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Hemocyanins (Hcs) are high molecular weight copper proteins which play the role of oxygen-carriers in the hemolymph of some Molluscs and Arthropods. We performed SAXS experiments on hemocyanins from both mollusc and arthropod species with the aim to increase information on their quaternary structure. The molecular architecture of arthopod Hcs is much better described and it results from the polymerisation of the 16 S basic unit defined by x-ray crystallography. We collected data at different protein concentrations on the *Carcinus maenas* and *Limulus poliphemus* Hcs (Arthropods) in various aggregation states (5S, 16S, 24S, 36S, 48S) and on the *Octopus vulgaris* and *Rapana thomasiana* Hcs (Molluscs). The radii of gyration have been calculated and correlated with those predicted from electron microscopy. The structural information obtained from electron microscopy have been used to simulate the scattering curves of the various aggregates of Hcs from Mollusc and Arthropod species.

STRUCTURE AND DYNAMICS OF THE HAEM POCKET IN *Methylophilus methylotrophus* CYTOCHROME *c"*, A HAEM PROTEIN COUPLING ELECTRON AND PROTON TRANSFER

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Cytochrome *c"* is an unusual soluble monohaem cytochrome (15 kDa) isolated from the obligate methylotroph *Methylophilus methylotrophus* [1]. NMR and ultraviolet/visible spectroscopies have shown that the haem undergoes a redox-linked spin-state transition, going from a low-spin state in the oxidized form to a high-spin state in the reduced form. The axial ligands are two histidine residues in the oxidized form, determined using MCD analysis [2], and a single histidine residue in the reduced form determined by NMR. Furthermore, MCD and EPR studies provided evidence for a near-perpendicular orientation of the two axial histidine residues in the oxidized form [2]. The midpoint redox potential of this haem protein shows a strong pH dependence (redox-Bohr effect) in the physiological pH range. The pH dependence of the midpoint redox potential has been analyzed in terms of a model that considers two ionizing groups with pK_a values which change with the redox state of the protein [3]. It was determined, that the group principally responsible for the redox-Bohr effect exhibited by cytochrome *c"* is the τ-NH of the axial histidine that becomes detached in the reduced state.

A complete assignment of the proton resonances of the haem, haem-ligands and other residues in the haem pocket was achieved in the oxidized form, using standard COSY, TOCSY and NOESY spectra. ^{13}C-^{1}H-NMR shift correlation (HMQC) enabled assignment of all the haem β-carbon resonances and the ^{13}C paramagnetic shifts have near axial asymmetry, in agreement with the EPR results.

In order to obtain information on the dynamics of the haem environment, measurements of $^{1}H/^{2}H$ exchange rates of amide protons located in the haem cavity were performed and showed substantial asymmetry on the mobility of the protein in the two haem faces. All of the NMR information together with protein sequencing data lead to the conclusion that the redox-linked protonation occurs via a channel running through the haem cleft on the haem face opposite to that containing the histidinyl ligand that becomes detached upon reduction of the iron atom [4].

[1]Santos, H. and Turner, D.L. (1988) *Biochim. Biophys. Acta* **954**, 276-286.
[2]Berry, M., George, S., Thomson, A., Santos, H. and Turner, D. (1990) *Biochem. J.* **270**, 413-417.
[3]Costa, H.S., Santos, H., Turner, D.L. and Xavier, A.V. (1992) *Eur. J. Biochem.* **208**, 427-433.
[4] Costa, H.S., Santos, H. and Turner, D.L. (1993) *Eur. J. Biochem.* **215**, 817-824.

Kinetic Studies of Cr(VI) / Thiol Reduction Reactions

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A better understanding of the intracellular reduction of Cr(VI) by thiol containing components is a main goal when studying the mechanisms of chromium toxicity [1,2]. Kinetic behaviour of Cr(VI) and intermediate formation and decomposition reduction reactions are important objectives to attain in this subject.

Kinetic studies of chromate reduction by glutathione were carried out, at neutral pH. Strong evidence for a biphasic process was obtained. The results were treated by a non-linear least-squares fitting, assuming two consecutive first order reactions. Rate constants were calculated for both processes.

The same reduction reactions were followed by stopped-flow kinetics, using cysteine as the reductant. In this case, the reaction "pattern" appears to be a simple first-order process.

In addition, thioester intermediates were detected and identified and their formation and decomposition kinetic rate constants were determined (for glutathione by UV/Vis spectrophotomety and for cysteine by stopped-flow kinetic methods).

A correlation between the kinetic rate constants of the chromate reduction and thioester formation and decomposition were found and is discussed as well.

References:

[1] - Connett, P. H. and Wetterhahn, K. E., *J. Am. Chem. Soc.*, 1985, **107**, 4282-4288

[2] - O'Brien, P., Wang, G. and Wyatt, P. B., *Polyhedron*, 1992, **11(24)**, 3211-3216

COMPLEXATION OF BRAIN AND EYE MELANIN WITH IRON AND COPPER IONS

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Melanin is a heteregenous polymer of diphenol or aminophenol origin, in which various subunits are linked by different bonds. Due to its amorphous character and insolubility, present knowledge about molecular structure, biogenesis and biological role(s) of this intractable pigment is far from satisfactory. This is particularly true in case of non-cutaneous melanin which, in human, is present in the inner ear, neuroepithelial cells of the eye and in certain dopaminergic neurons of the midbrain. It is believed that among physicochemical features of melanin, its metal-ion binding and redox properties are biologically most relevant.

We have measured total concentration of iron (and other metals) in pigmented and non-pigmented areas of the human brain by Total Reflection X-Ray Fluorescence and compared the results with those obtained by Electron Spin Resonance (ESR) determination of iron(III) at 77 K. The data indicate that, while high iron content could be detected in several brain tissues, the strongest ESR signal at $g = 4.3$, characteristic for high-spin ferric complexes, was predominantly seen in the distinctively pigmented *substantia nigra*. We also observed that the intensity of the ESR signal was reduced by treatment of the brain tissue that stimulated partial release of bound-to-neuromelanin metal ions. Thus, it appears that the ESR signal at $g = 4.3$, detected in human *s.nigra* is, to a large extent, determined by the amount of iron accumulated in the neuromelanin. Similar observations were made in a study of retinal pigment epithelial (RPE) cells, isolated from human and bovine eyes. It has been established that the detectable ESR signal of high-spin Fe(III) was mainly due to its complexes with RPE melanin. Presence of iron in the RPE melanin granules has been directly determined by Energy Dispersive X-ray microanalysis. Using this sensitive method, we were able to observe distinct changes of metal content in RPE malnosomes with aging and as a result of their *ex vivo* photobleaching.

The effect of oxidative bleaching of melanin on its metal-ion binding ability was further analyzed *in vitro* by ESR spectroscopy, using synthetic melanins, and Cu(II) and Fe(III) as extrinsic molecular probes. The data suggest that such a chemical modification of melanin may lead to reduction of its antioxidant activity which is chiefly determined by melanin's capacity to sequester redox-active metal ions.

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND DNA CLEAVING ABILITY OF NEW COVALENTLY-LINKED DINUCLEAR RUTHENIUM BIPYRIDYL AND O-PHENANTHROLYL COMPLEXES.

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Institut de Physique Nucléaire, IN2P3, 91405 Orsay Cedex, France.

Using covalently linked bipyridines as ligands, we prepared binuclear ruthenium(II) complexes with general formula

$N\,N$ = o-phen. or 2,2'-bipy.

$R=(CH_2)_{10}$

$= -CH_2NH(CH_2)_8NHCH_2-$

$= -CH_2NH(CH_2)_3\,N\,(CH_2)_3\,NHCH_2-$
$\qquad\qquad\qquad CH_3$
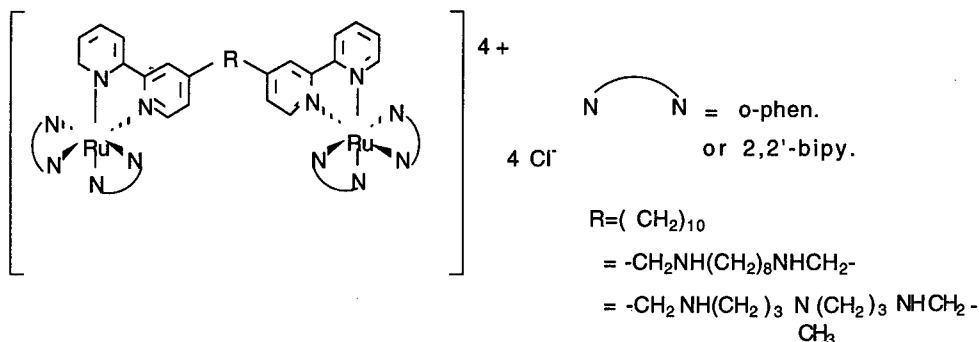
The complexes were fully characterized by usual methods and in particular by Phase Desorption Spectrometry.

Their spectroscopic and photophysical properties demonstrate the absence of electronic coupling between the two Ru sites.

With L = 2,2'-bipydine, the nature of R does not affect significantly these properties but some variations are observed with L = o-phenanthroline mostly in the presence of DNA.

The cleaving ability of these complexes after irradiation on plasmid DNA will be presented.

I. Sasaki, M. Imberdis, A. Gaudemer, B. Drahi, D. Azhari, E. Amouyal, New J. Chem., 1994, in press.

Kinetic Studies on the Type II Copper Protein Galactose Oxidase with Small Inorganic Complexes.

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Galactose Oxidase (GO) is a type II Cu protein secreted extracellularly by the wood rot fungus *Fusarium dendroides*. It has more recently been expressed in *Aspergillus niger* which is the source in these studies. Its precise physiological function is uncertain. GO is a highly basic protein (pI=12) of molecular weight 68 kDa. It consists of a single polypeptide chain of 639 amino acid residues contained in three domains, and a single Cu atom at the active site. The crystal structure of GO has been determined to a resolution of 1.7Å (1). GO catalyses the oxidation of a wide range of primary alcohols to the corresponding aldehydes. This oxidation is highly stereo specific. A two electron redox capacity is achieved by involvement of the Cu^{II}/Cu^{I} and Tyr·/Tyr redox changes.(2)

$$\underset{\text{oxidised}}{Cu^{2+}\text{-Tyr}\cdot} \rightleftharpoons \underset{\text{semi}}{Cu^{2+}\text{-Tyr}} \rightleftharpoons \underset{\text{reduced}}{Cu^{+}\text{-Tyr}}$$
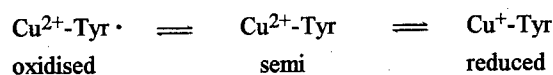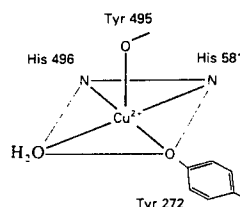
Work to date has been concerned with the stopped-flow redox interconversion of the semi and oxidised forms with $[Fe(CN)_6]^{3-}$ and $[Co(phen)_3]^{3+}$ as oxidants and $[Fe(CN)_6]^{4-}$ as reductant. Semi GO shows a remarkable decrease in reactivity from $4300M^{-1}s^{-1}$ at pH 6 to close to zero at pH 9 (pK_a 7.6). This process may correspond to acid dissociation of the Cu bound water molecule making the association of the negatively charged oxidant less favourable. In contrast, the oxidation of semi with $[Co(phen)_3]^{3+}$ shows the reverse effect with rate constants increasing with pH . Reduction of oxidised GO with $[Fe(CN)_6]^{4-}$ is also pH dependent but the pK_a is now <7. This is an unusual shift in view of the retention of the Cu(II) oxidation state suggesting that other explanations should not be excluded. Further studies in which the Cu bound water molecule was replaced with N_3^- and its effect on redox behaviour will also be considered.

1. N.Ito *et al*, *Nature.*,1991, **350**, 87.
2. M.M.Whittaker *et al*, *J. Biol. Chem.*,1989, **264**, 7104.

THE STRUCTURAL ROLE OF IRON IN HEME PROTEINS.

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The structural role played by iron in heme proteins differs from that of other metals in one important respect. While most metals are chelated only by protein side-chains, the heme iron has already committed four of its six coordination sites to the porphyrin. This ligation restricts the motional freedom of the metal and modulates its donor and acceptor properties. In the special case of cytochrome c this modulation can be specifically studied because the covalent attachment of the porphyrin to the protein imposes a further motional restriction that makes the iron a true component of the whole structure. A striking consequence of this chelation is the enhancing of the iron affinity for a thioether sulfur, particularly so in the reduced state. Three major factors modulate this specific affinity: the stereochemistry of the trans- imidazole ligand; the presence of point charges close to the heme; and the effect of interior water dipoles. Studies with cytochrome c mutants are beginning to reveal how these structural parameters affect the stability of the iron-sulfur bond and of the whole molecule, and the redox potential of the iron.

Photoinduced Electron Transfer Studies in Metalloporphyrin Model Compounds

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In order to better understand the dynamics and mechanism of intramolecular photoinduced electron transfer reactions, a series of new metalloporphyrin dimers has been synthesized. Utilizing previously described nucleophilic substitution reactions[1] on porphyrins possessing only a single *meso*-pentafluorophenyl group we have prepared a set of building blocks suitable for the sequential synthesis of porphyrin dimer systems. Our new synthetic methodology allows the systematic modification of several variables in the diporphyrin system including type and length of spacer, metal center, and potential difference between the donor and acceptor metalloporphyrin. We now report the preparation of a series of (Zn^{II} - Fe^{III}) bisporphyrin hybrid complexes which are rigidly connected by nonaromatic diamine linkers.

X=H,Cl,F; Y=H,F; Z=H,Cl
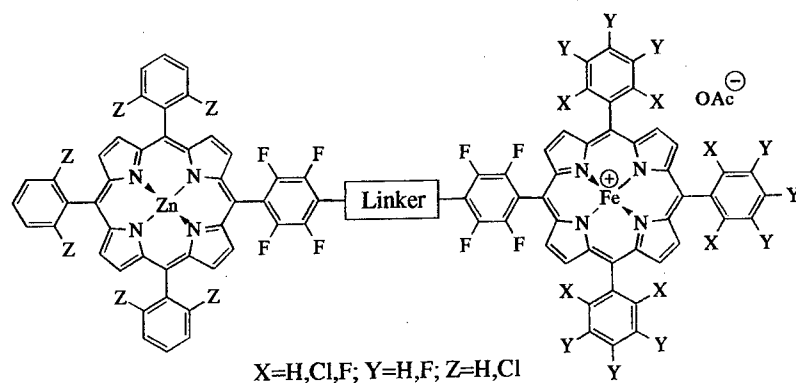
The mechanism and dynamics of the photoinduced electron transfer reactions in these model systems has been investigated by time resolved fluorescence technique in the picosecond time domain.

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[1] P. Battioni; O. Brigaud; H. Desvaux; T.G. Traylor, *Tetrahedron Lett..*, **32**, 2893 (1991).

Cytochrome *c* nitrite reductase from *Sulfurospirillum deleyianum*: Molecular parameters and active site structure

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Oxidized nitrogen compounds function as electron acceptors for anaerobic growth of prokaryotes and are finally reduced to N_2 (denitrification) or to NH_4^+ (nitrate ammonification). The respiratory nitrite reductases from nitrate-ammonifying *Sulfurospirillum deleyianum* and *Wolinella succinogenes* are *c*-type cytochromes that catalyze the six-electron reduction of nitrite to ammonia:

$$NO_2^- + 6\,e^- + 8\,H^+ \longrightarrow NH_4^+ + 2\,H_2O$$
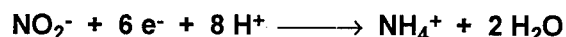
From a comparison of the molecular and EPR properties of several cytochrome *c* nitrite reductases it was concluded that these proteins constitute a group of homologous enzymes with six covalently bound heme groups. In addition, the enzymes tend to form aggregates *in vitro*.

The membrane fraction of *S. deleyianum* and *W. succinogenes* gave a high molecular weight form (M4-NiR, 245 kDa, $\geq$17 heme and Fe/M_r) and a low molecular weight form of the nitrite reductase (M1-NiR, 55 kDa, 3.7±0.3 heme and Fe/M_r); the soluble fraction gave only the low molecular weight form (S1-NiR, 55 kDa, 3.2±0.4 heme and Fe/M_r). Thus, S1- and M1-NiR are tetraheme proteins. The various forms of NiR exposed identical epitopes according to Western blot analysis with antiserum raised against S1-NiR. For the first time, M4-NiR, probably the physiologically relevant form, was shown to be a heterooligomeric complex composed of four identical subunits of M_r 55 kDa plus at least one subunit of a 22 kDa cytochrome *c*, the latter proposed to be the physiological electron donor to nitrite reductase. An identical structure and molecular composition was found for M-NiR of *W. succinogenes*. EPR spectroscopy revealed resonances in the as isolated M4-NiR at *g*=4.8, and in the M4-NiR at intermediate redox potentials at *g*=3.1. Therefore, these resonances that were not observed neither for S1- nor for M1-NiR, are considered to originate from the 22 kDa cytochrome *c* subunit of M4-NiR. Redox-cycled NiR showed Fe(III) resonances that were not observed for the as isolated NiR. Reduction of M1- and M4-NiR followed by stopped-flow spectrophotometry exhibited a biphasic time course for both forms with differences in heme reactivity. These data will be interpreted in terms of a mechanistic model.

NUCLEAR MAGNETIC RESONANCE AND KINETIC STUDIES
OF FERREDOXIN FROM *CLOSTRIDIUM PASTEURIANUM*

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Recent studies have found that both protein folding and degree of hydrogen bonding dictate redox properties and electronic states of Fe-S clusters.[1,2] By specific variation of amino acid residues with site-directed mutagenesis, residues which are essential to the redox chemistry of these proteins can be identified.

The ferredoxin from *Clostridium pasteurianum* (CpFd) is a 55 amino acid residue protein which contains two Fe_4S_4 clusters bound to the protein backbone by eight cysteinyl residues.[3] Mutant forms of CpFd have allowed the sequence-specific NMR assignment of all cysteinyl spin systems. These results complement those reported previously using a different approach.[4]

Progress toward sequence-specific assignment of non-cysteinyl spin systems[5] used to identify sites crucial to electron transfer[6] will be reported.

1.   Backes, G.; Mino, Y.; Loehr, T. M.; Meyer, T. E.; Cusanovich, M. A.; Sweeney, W. V.; Adman, E. T.; Sanders-Loehr, J. *J. Am. Chem. Soc.* **1991**, *113*, 2055.

2.   Langen, R.; Jensen, G. M.; Jacob, U.; Stephens, P. J.; Warshel, A. *J. Biol. Chem.* **1992**, *267*, 25625.

3.   Adman, E. T.; Sieker, L. C.; Jensen, L. H. *J. Biol. Chem.* **1973**, *248*, 3987.

4.   Bertini, I.; Capozzi, F.; Luchinat, C.; Piccioli, M.; Vila, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 651.

5.   Scrofani, S. D. B.; Brownlee, R. T. C.; Sadek, M.; Wedd, A. G. *Biochemistry*, submitted.

6.   Armstrong, F. A.; Henderson, R. A.; Ong Wah Kim, H.; Sykes, A. G. *Biochem. Biophys. Acta* **1982**, *681*, 161.

SYNTHESIS AND PROPERTIES OF DINUCLEAR μ-OXO-μ-CARBOXYLATO IRON(III) COMPLEXES WITH A LABILE COORDINATION SITE, MODELS OF NON-HEME IRON PROTEINS

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The synthesis by self-assembly and spectroscopic characterization (UV-VIS, IR, RR, EPR, Moessbauer, NMR) of novel complexes containing a Fe_2O core with no, one or two bridge carboxylate, bidentate (bpy, phen) and labile monodentate (H_2O, MeOH) terminal ligands are reported.

The characteristics μ-oxo-μ-carboxylato complexes in hydroxylic solvents resemble those of hemerithrine and ribonucleotide reductase, but in non-hydroxylic solvents are very close to methane monooxygenase. On the base of Raman spectroscopy and magnetic measurements it is suggested that there is a strong intramolecular H-bond between bridging oxygen and cis-water in non-hydroxylic solvents.

An influence of substituents in terminal and bridge ligands on properties and catalytic activity of these complexes was studied.

An alkane (methane, ethane, hexane, cyclohexane and adamantane) oxidation by hydrogen peroxide and t-BuOOH catalyzed by these complexes was studied in MeCN. The nature of possible active intermediates and mechanism, and also a relation to methane monooxygenase catalytic cycle, will be discussed.

Synthesis and Properties of Nitro and Nitrosyl Complexes of Manganese and Iron Group Metallophthalocyanines

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In recent years nitric oxide has been the subject of much interest and debate due to its expanding repertoire of important bioregulatory functions. As the NO activated processes seem to require the presence of a heme moiety the complex formation of NO with heme proteins and $Fe^{2+/3+}$ porphyrinic substitutes has been investigated pointing to the extraordinary affinity of NO for Fe(II) heme and to its labilizing effect on proximal ligands. Here we report on nitro and nitrosyl containing phthalocyanines (Pc). These chelate complexes are structurally related to porphyrines but the inverted sequence and greater separation of the first two π-π^* transitions makes the resonance Raman effect a particularly useful probe.

Dinitro complexes of the iron group metallophthalocyanines ($[M(NO_2)_2Pc^{2-}]^-$) are obtained by the reaction of the halo- or dihalo metallophthalocyanines with nitrite in alkaline acetone or in molten $(^nBu_4N)NO_2$. These complexes react in acid solution in the presence of NO_2^- presumably via an inter-ligand reaction involving the coordinated and free nitrite ion thereby precipitating nitrosyl ($[M(NO)Pc]$) or dinitrosyl complexes ($[M(NO)_2Pc]$). Di(nitrosyl)phthalocyaniantoiron must be prepared at low temperature, but is quite stable as a solid at room temperature. $[Mn(NO_2)_2Pc]^-$ is unstable and in the presence of $[BH_4]^-$ $[Mn(NO_2)(NO)Pc]^-$ is obtained, dissociating in CH_3OH to yield $[Mn(NO)Pc]$.

The coordination chemistry of the nitrosyl complexes has been investigated and different hexa-coordinated complexes are isolated. Vibrational spectroscopy combined with electronic absorption spectra and magnetic susceptibility data has been used to determine the structural and bonding properties of the M-N-O moiety.

The 'Self-Enzymic' Properties of Adenosine 5'-Di- and 5'-Triphosphate in Their Metal Ion-Promoted Hydrolysis

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Are there low-molecular-weight biomolecules with enzymic properties, primitive ancestors of today's enzymes? Could an interplay between such molecules have promoted certain reactions beyond the chemical 'noise' present on the early earth,[1] thus giving rise to selectivity and a primitive metabolism? I would like to suggest[2] that with certain metal ions adenine-nucleotides are such substances.

Mechanistic studies revealed[3] that the dephosphorylation of ATP with ions (M^{2+}) such as Zn^{2+}, Cd^{2+}, or Cu^{2+} proceeds via dimeric species, i.e., $[M_2(ATP)]_2(OH)^-$ or $[M_2(ATP)]_2$ depending on pH; for Cu^{2+} at pH > 6.5 also the $[Cu(ATP)]_2(OH)^{5-}$ dimer was identified. These dimers occur in low concentrations and involve purine stacking and a M^{2+}/N-7 interaction. In other, considerably less reactive triphosphate monoesters with a non-coordinating organic residue, including pyrimidine-nucleoside 5'-triphosphates ($PNTP^{4-}$), monomeric $M_2(PNTP)(OH)^-$ species govern the reactivity.[3] The reason for the high reactivity of the $[M_2(ATP)]_2$ dimers is that one of the two ATPs takes over a structuring role by facilitating the shift of one M^{2+} along the triphosphate into an α,β position, promoting thus the split between the β and γ groups (the latter also carries a M^{2+}); thus ATP acts as its own hydrolytic 'enzyme'.[2,3]

The structuring ATP may be replaced (at neutral pH) by AMP,[3] but not by TuMP (7-deaza-AMP) which proves that N-7 is crucial. With Cu^{2+} the reaction most probably proceeds via $Cu_3(ATP)(AMP)(OH)^-$ and $Cu_2(ATP)(AMP)(OH)^{3-}$ species.[2]

Corresponding observations have now also been made[4] with Cu^{2+}/ADP at pH 5.5: (*i*) the reaction proceeds via a dimer, (*ii*) the highest reactivity occurs with the ratio $Cu^{2+}:ADP = 2:1$, and (*iii*) addition of AMP enhances again the reactivity.

These indicated self-promoting properties in the presence of metal ions are also expected for transphosphorylations from ATP or ADP not only to water, but to other receptors as well. Thus, certain metal ion-adenine nucleotide systems could have been favored on the early earth,[2] allowing them to stick out of the chemical 'noise' and giving rise to selectivity in a primitive metabolism without a genetic code.

(1) M. Mitchell Waldrop, *Science 246*, 1248-1249 (1989). (b) C. de Duve, "Blueprint for a Cell: the Nature and Origin of Life", N. Patterson Publ., Burlington, NC, 1991.
(2) H. Sigel, *Inorg. Chim. Acta 198-200*, 1-11 (1992).
(3) H. Sigel, *Coord. Chem. Reviews 100*, 453-539 (1990).
(4) H. Sigel, M. Bastian, and S. S. Massoud, results to be published.

MANGANESE POLYNUCLEAR COMPLEXES WITH UNSYMMETRICAL PENTADENTATE SCHIFF BASES AS MODELS FOR THE OEC OF PHOTOSYSTEM II. SYNTHESIS, REACTIVITY, MOLECULAR STRUCTURE AND MAGNETIC PROPERTIES.

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Among the metalloproteins and enzymes including manganese, the dioxygen evolving complex of photosystem II (OEC) has been the most extensively studied. A variety of studies indicate this site to be a Mn_4 agregate operating on the donor site of PSII and a number of models have been synthesized with dinuclear and higher nuclearity manganese compounds. In this study, we describe polynuclear manganese complexes with unsymmetrical dinucleating Schiff base ligands affording a N_2O_3 donor set, 1,4-bis(X-salicylideneamino)-2-butanol (X=H, L^1; X=5-NO_2, L^2; X=5-Cl, L^3; X=5-Br, L^4), 1,4-bis(2-OH-naphtalideneamino)-2-butanol (L^5) and 1,4-bis(benzophenilidene-amino)-2-butanol (L^6). According to the reaction conditions the following types of complexes can be obtained: $(Mn^{II}_2L(\mu\text{-}OAc)(solv.)_m)_n$ (A), $(Mn^{II}_2L(\mu\text{-}OMe)(solv.)_m)_n$ (A'), $(Mn^{II}L(solv.)_m)_n$ (B) (solv. refers to MeOH or H_2O). IR spectroscopy indicates that phenolic (and alcoholic in A and A') oxygen and imine nitrogen atoms are coordinated to the Mn ions. Powder and frozen solutions EPR spectra of A and A' species show a broad band centred at g≈2, while EPR spectra of B species exhibit at least 10 fine-structure resonances splitted into a 11-line pattern characteristic of Mn pairs, in frozen solution. The X-ray molecular structure of $[Mn^{II}_2L^1(\mu\text{-}OMe)(MeOH)]_2$, 2MeOH crystals (A') obtained from methanolic solutions of $(Mn^{II}_2L^1(\mu\text{-}OAc)(solv.)_m)_n$ (A) suggests that methanolate bridges allow formation of a polynuclear structure more stable than the initial one (including acetate bridges), probably due to steric constraints. Reaction of complexes A and A' with dioxygen or direct synthesis from $Mn(OAc)_3$ yields complexes of general formula $(Mn^{III}_2L(\mu\text{-}OR)_3(solv.)_m)_n$ or $(Mn^{III}_2L(\mu\text{-}OR)(\mu\text{-}O)(solv.)_m)_n$, suggesting that, as a consequence of the shorter Mn-L and Mn-O-Mn distances, axial positions are occupied by the ligand leading to a better stabilization of Mn^{III} species. The synthesis, reactivity, structural features and magnetic properties of these new polynuclear manganese complexes will be presented.

Sequence-Selective Binding of Mn(II), Zn(II), and Hg(II) Ions to DNA Oligomers Studied by NMR Spectroscopy

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The nucleobase affinities towards metal ions are modified when incorporated in a duplex DNA matrix. The melting and renaturing behaviour of native DNA in the presence of rather high concentrations of various divalent metal ions show an interesting variation through the series of metals [1]. These observations could be explained in terms of a varying degree of affinity towards the phosphate backbone and the base nitrogens. In the lecture NMR studies of the interaction between metal ions and short oligodeoxyribonucleotides are presented. The focus will be on certain transition metal ions which are expected to interact with DNA in a site-specific manner. In order to elucidate the rules for sequence-selectivity several different oligonucleotides have been titrated with Mn(II), Zn(II) and Hg(II) salts monitored by ^{1}H NMR spectroscopy [2,3,4]. Recently a series of titration experiments on oligomers have also been carried out involving different palladium species (Pd^{2+}, $Pd(en)^{2+}$, and $Pd(dien)^{2+}$). The results will be dicussed in relation to :

1. The possibility of preferential metal ion binding sites on DNA
2. Metal ion induced structural changes of duplex DNA, local variation
- as well as overall changes in secondary structure.
3. Differences in binding mode between various metal ions.

The two main factors which determine the preferential binding sites are:
i) the coordination geometry of the metal ions and ii) the magnitude of the partial negative charge on the potential binding sites of the nucleobases themselves. The fluctuation in nucleophilicity of donor sites is a consequence of subtle sequence-dependent conformational changes that alter base stacking.

References

1. Eichhorn, G. L. and Shin, Y. A., (1968) *J. Am. Chem. Soc.* 90, 7323-7328.
2. Frøystein, N. Å. and Sletten, E. (1991) *Acta Chem. Scand.* 45, 219-225.
3. Frøystein, N. Å., Davis, J. T., Reid, B. R. and Sletten, E. (1993)
   Acta Chem. Scand. 47, 649-657.
4. Frøystein, N. Å. and Sletten, E. (1994) *J. Am. Chem. Soc.*(in press)

TRANSITION METAL COMPLEXES OF 2-OXIMINOCARBOXYLIC ACIDS -

ANALOGUES OF AMINOACIDS.

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Some years ago we have begun a detailed investigation of this new type of oximes, which can be considered as structural analogues of the 2-aminoacids. 2-Oximinicarboxylic acids — RC(NOH)COOH, (R-aliphatic radical) may be considered as di-basic acids. It was shown that the deprotonation of the carboxyl group by bivalent metals leads to uncharged compound of the type $M(HQ)_2 *nL$ (H_2Q = 2-oximinopropionic (H_2A) and 2-oximino-3-phenylpropionic (H_2B) acids; $L=H_2O$, NH_3, Py, imidazole; M(II)=Co, Ni, Cu, Zn). Their structures have been deduced on the base of electronic, IR, PMR spectra and X-ray analysis, as well as their solution conductivity. From our point of view these ligands and their complexes can be useful as bioactive materials. Some of them are constituent of growth factors, another-stimulates synthesis of antibiotics snd so on.

Mixed complexes of Co (III) with H_2A or H_2B acid and different amine (imidazole, benzimidazole, pyridine, β-picoline, γ-picoline) are obtained. The organic ligands behave as O,N- donors via the carboxyl oxygen and the oxime nitrogen atoms. A trans-octahedral structure has been assigned to the bis(2-oximinocarboxylato)bis(amine)cobalt (III) on the basis of PMR data.

The crystal and molecular structures of the complexes trans-[bis (2-oximinopropionato)bis-(imidazole)]- and trans-[bis(2-oxi minopropionato}bis(pyridine)] cobalt(III) were determined. The coordination sphere around Co is pseudo-octahedral with the 2-oximinopropionato ligands occupying four equatorial positions and the amines in axial positions.

COMPLEXES OF HUMAN LACTOFERRIN WITH VANADIUM IN OXIDATION STATES +3, +4 AND +5

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Human lactoferrin binds vanadium in the +3, +4 and +5 oxidation states with a stoichiometry of two metals: mole of protein. Binding occurs in the two specific metal binding sites and formation of the complexes was followed by UV difference spectroscopic titrations. The redox stability of the three vanadium-lactoferrin complexes was investigated by ESR spectroscopy and the vanadium(III)-lactoferrin complex was found to be unstable with respect to vanadium(IV)-lactoferrin. This is in contrast to the previously published (1) transferrin system where the vanadium (III) species was more stable. The vanadium-lactoferrin systems were modelled into the specific binding sites of diferric and dicupric lactoferrin and these results will be discussed.

1   I. Bertini, C. Luchinat and L. Messori, *J. Inorg. Biochem.,* **25** (1985) 57.

^{1}H Assignments of the oxidized HiPIP from *Chromatium vinosum*

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An almost complete proton resonance assignment of the oxidized high-potential iron-sulfur protein (HiPIP) from *Chromatium vinosum* was obtained by employing conventional 2D NMR routines. Hereby, we were able to identify the through-bonds and through-space connectivities, which are the prerequisite for the determination of the 3D solution structure. With the aid of the ^{1}H assignments of the oxidized protein in the previous reports more than 1400 NOE cross-peaks could be identified for 84 out of 85 assigned residues. Additional assignments were obtained using 1D NOE, 2D HMQC, 2D NOESY and 2D TOCSY experiments in H_2O and D_2O with different mixing times. Ser-79 remains unassigned. The sequence specific assignment in the fingerprint region yields eight segments. Breaks in this sequence are mostly caused either by prolines or by paramagnetically shifted protons in the vicinity of the iron-sulfur cluster. The largest segment ranges from PRO-3 to ARG-33, other segments are SER-1-ALA-2, GLY-35-LEU-36, PRO-38-ASN-45, GLN-50-CYS-63, GLN-64-LEU-65, PRO-67-TRP-76, THR-81-GLY85. The $d_{\alpha N}$ connectivities of the cysteine residues between HIS-42-CYS-43, CYS-46-GLN-47 and GLY-62-CYS-63 were found in the 2D NOESY map. Moreover we were able to identify most of the aromatic protons of TYR-19, HIS-42, PHE-48, PHE-66, TRP-60, TRP-76 and TRP-80.

Acid–Base and Metal-Ion–Binding Properties of 2'-Deoxycytidine 5'-Monophosphate (dCMP^{2-}) Coordinated to *cis*-Diammine-Platinum(II)

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Most biological reactions involving nucleotides and nucleic acids depend on metal ions; consequently, the coordination chemistry of these ambivalent ligands with labile[1] as well as inert[2] metal ions is intensively studied. So far little information exists about the mutual effects different metal ions bound to the same nucleotide are exerting on each other or about the effect of a bound metal ion on protonation/deprotonation reactions. The acidity constants for $H_2(dCMP)^{\pm}$ in aqueous solution are $pK_{a/1} = 4.46 \pm 0.01$ [deprotonation of $H^+(N-3)$] and $pK_{a/2} = 6.24 \pm 0.01$ [deprotonation of $-P(O)_2(OH)^-$] (25°C; $I = 0.1$ M, $NaNO_3$). The latter value may be compared with the situation when two $H(dCMP)^-$ ions are coordinated via N-3 to cis-$(NH_3)_2Pt^{2+}$, which results in $H_2[cis$-$(NH_3)_2Pt(dCMP)_2]$, abbreviated as $H_2 \cdot Pt(dC)_2$. Deprotonation of its two $-P(O)_2(OH)^-$ residues occurs with $pK_{a/1} = 5.73 \pm 0.02$ and $pK_{a/2} = 6.47 \pm 0.02$. The difference $\Delta pK_a = 0.74 \pm 0.03$ is slightly larger than the statistically expected value of 0.6, indicating a small mutual influence of the two $-P(O)_2(OH)^-$ sites. Furthermore, the average pK_a of 6.10 [= (5.73+6.47)/2] being about 0.14 pK_a units below $pK^H_{H(dCMP)}$ (= 6.24) indicates a small acidifying effect of Pt^{2+} at the N-3 sites on these two residues. Similarly, the stability constants of the $M[H \cdot Pt(dC)_2]^+$ complexes of Mg^{2+} and Zn^{2+} (log $K^{Mg}_{exper} = 1.31 \pm 0.15$; log $K^{Zn}_{exper} = 1.86 \pm 0.05$) are to about the same extent lower than expected from the linear log $K^M_{M(L)}$ *versus* $pK^H_{H(L)}$ relationship[3] and $pK_{a/1} = 5.73$ (log $K^{Mg}_{calc} = 1.47 \pm 0.03$; log $K^{Zn}_{calc} = 1.96 \pm 0.06$). Upon deprotonation of both phosphate residues the repulsive effect of Pt^{2+} is slightly overcompensated by the attracting effect of the second $-PO_3^{2-}$ group, giving slightly larger stability constants than expected based on the basicity of the other $-PO_3^{2-}$ residue (log $K^{Mg}_{Mg[Pt(dC)_2]} = 1.98 \pm 0.07$; log $K^{Zn}_{Zn[Pt(dC)_2]} = 2.74 \pm 0.06$).

(1) H. Sigel, *Chem. Soc. Reviews* **22**, 255-267 (1993). (2) B. Lippert, *Biometals* **5**, 195-208 (1992). (3) H. Sigel, S. S. Massoud, and N. A. Corfù, *J. Am. Chem. Soc.* **116**, 2958-2971 (1994).

POTENTIOMETRIC AND SPECTROSCOPIC STUDIES ON COPPER(II) PEPTIDE COMPLEXES

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Complex formation processes between copper(II) and oligopeptides have already been studied by a number of various techniques and it has been well identified that terminal amino and subsequent amide nitrogens are the main metal binding sites. The deprotonation and coordination of the amide nitrogens, however, largely depend on the other donor groups present in the molecules and may yield, in addition to the common 1:1 complexes, various bis complexes and some other species with different binding sites. The combined application of potentiometric and spectroscopic (EPR and UV-visible) studies makes it possible to identify the basic coordination sites and to determine their spectral parameters. The ligands covered the most common derivatives of glycine (N-acetyl-glycine, glycineamide, glycyl-sarcosine, diglycine, triglycine, tetraglycine, diglycine-amide, glycyl-β-alanine and β-alanyl-glycine) having the carboxylate, carbonyl, amino and amide groups in different chemical environment.

The results were applied for the elucidation of the complex formation processes of various oligopeptides containing additional oxygen (carboxylate groups of aspartyl and glutamyl or phenolate of tyrosyl), nitrogen (amino group of lysyl residues) or sulfur (thioether or disulfide) donor atoms in the peptide molecules.

Molybdenum(VI), (V) and (IV) Complexes with Tridentate S$_2$N- and SNO- Donor Systems

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Molybdenum plays an essential role in the active sites of many hydroxylases such as sulfite oxidase and xanthine oxidase. During the enzymatic reaction the molybdenum center cycles through the formal oxidation states (VI), (V) and (IV). Structural investigations (EXAFS, EPR) on sulfite oxidase led to the proposed minimal coordination sphere at the active center of Mo(VI)O$_2$S$_{2,3}$(N/O) in the oxidized and Mo(IV)OS$_{2,3}$(N/O) in the fully reduced form[1].

Figure 1: MoO$_2$(2-map-S-bnzdtc)*pic

We therefore synthesized Mo-complexes in the three relevant oxidation states with a S$_2$N- resp. SNO-coordination sphere. The ligands which we utilized were obtained by condensation of substituted salicylaldehydes, 2-mercaptobenzaldehyde and 2-mercaptoacetophenone with 4-substituted thiosemicarbazides or S-benzyl-dithiocarbazate. Reaction of S-benzyl-3-(2-mercaptophenylmethyl)methylenedithiocarbazate (2-map-S-bnzdtcH$_2$) with MoO$_2$(acac)$_2$ in donor solvents yields, for example, the mononuclear Mo(VI)complex MoO$_2$(2-map-S-bnzdtc)*pic shown in Figure 1, whereas the Mo(IV) species are obtained by subsequent reduction with PPh$_3$. Reaction with the precursor [Et$_4$N][MoO(SPh)$_4$] results in the Mo(V) complexes. In some cases binuclear μ-oxo-Mo(V)complexes are formed by the reaction of the thiolate ligands with MoO$_2$(acac)$_2$ as the major product.

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[1] G.N. George, C.A. Kipke, R.C. Prince, R.A. Sunde, J.H. Enemark, S.P. Cramer, Biochem. **1989**, *28*, 5075

Molecular Properties of the Dissimilatory Sulfite Reductase from *Desulfovibrio desulfuricans* (Essex): Localization and Purification of the 11 kDa protein component

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Desulfoviridins are siroheme and Fe-S centres containing sulfite reductases involved in the dissimilatory reduction of sulfite by sulfate-reducing bacteria. Recently, Pierik et al. [1] described a hitherto unnoticed 11 kDa protein component of desulfoviridin from *Desulfovibrio vulgaris* (Hildenborough) that they described as γ-subunit of an $\alpha_2\beta_2\gamma_2$ multimer. However, Karkhoff-Schweizer et al. [2] showed that the expression of the gene encoding for the so-called γ-subunit of desulfoviridin from *D.vulgaris* and of the α- and β–subunit genes are not coordinately regulated.

During gel filtration of desulfoviridin from *D.desulfuricans* (Essex) under non-denaturing conditions, we isolated a 11 kDa protein showing cross-reactivity with antibodies raised against the γ-subunit of desulfoviridin from *D.vulgaris*. Herein we report on localization and purification of the 11 kDa protein from *D.desulfuricans*. A physiological function of this protein will be discussed.

[1] A.J.Pierik, M.G.Duyvis, J.M.L.M. van Helvoort, R.B.G.Wolbert and W.R.Hagen, *Eur.J.Biochem.* 205, 111-115 (1992)

[2] R.Karkkoff-Schweizer, M.Bruschi and G.Voordouw, *Eur.J.Biochem.* 211, 501-507 (1993)

Computer Simulation Studies of Zinc Metalloenzymes

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Dipositive zinc, one of the more ubiquitous metal ions in biology, is known to be an integral component of a large number of enzymes and proteins involved in many aspects of metabolism. However, these systems are difficult to study by conventional molecular dynamics simulations due to the difficulty in treating the long-range electrostatic interactions which are evidently important in the presence of the zinc ion. In this presentation, recent progress in the study of zinc metalloenzymes by computer simulation will be discussed. The problem of treating long-range electrostatic interactions has been addressed by the development of an Extended Electrostatics model, a model which combines a standard pairwise additive scheme for spatially close interactions with a multipole approximation for the calculation of spatially distant interactions. The inclusion of long-range electrostatic interactions permits the development of a simple and general model for the zinc binding site. Examples to be discussed include molecular dynamics simulations of the zinc enzymes carboxypeptidase A and carbonic anhydrase.

Comparative Studies of Toxicity & Tissue Biodistribution of Two Mixed Copper Complexes: with Nalidixic Acid or Ciprofloxacin & 1,10-Phenanthroline.

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Copper in an essential metal, which carries out specific physiological roles. Almost all copper in living organisms is bound to specific copper proteins, as Superoxide Dismutase and Cytochrome-c-Oxydase. These, as other copper-dependent proteins are required by all cells, for normal metabolism and prevention of disease. Copper complexes may also have antiinflamatory, anticonvulsant, and anticancer activity. These considerations led us to suggest that copper complexes might be effective antineoplasic agents. Several copper complexes have been synthesized, with a general formula [Cu(N-N)(Antb)]X, where (N-N) is an aromatic diamine and Antb is the anionic form of a Quinolone (1). *in vitro* and *in vivo* experiments using [Cu(Phen)(Nal)]NO$_3$ (Phen is 1,10-Phenanthroline, Nal is Nalidixic Acid) have shown its amebicidal and bactericidal activity, as well as antineoplasic activity in the treatment of sarcoma S180 in mice. These results led us to study the toxicity and tissue biodistribution of these complexes, in normal mice. Several doses of [Cu(Phen)(Nal)]NO$_3$ and Cu(NO$_3$)$_2$ have been used, and copper content has been determined in liver, kidney, heart, and brain (2). Copper content is most important in liver when the 16mg complex/Kg weight is used, reaching 9 to 10 times the normal copper content, 3 hours after administration. Meanwhile, when the equivalent dose of Cu(NO$_3$)$_2$ is used, copper content is most important in kidney, reaching 4.5 to 5 times the normal copper content, 1 to 3 hours after administration. These results suggest that the complex and Cu(NO$_3$)$_2$ have different metabolism and therefore different effects. Toxicity and tissue biodistribution of these two species will be compared, as well as those of the Ciprofloxacin complex.

1. Mendoza-Díaz G., García-Nieto R.M., Gracia-Mora I., Arias-Negrete S., Ruiz-Ramírez L., Cozensa I.L., Moreno-Esparza R., Pannel K.H., and Cervantes-Lee F.; **Synthesis, Characterization, and Biological Activity of Some Mixed Complexes of Cu(II) and Zn(II) with Antibiotics of the Nalidixic Acid Family and (N-N) Ligands**; J. Inorg. Biochem., 43, [2-3], 640 (1991)

2. Súcar-Súccar S. and Mendoza-Díaz G.; **Toxicity and Tissue Biodistribution of Mixed Copper Complexes with Some Quinolone Drugs and (N-N) Donnors**; J. Inorg. Biochem. Abstracts for the Sixth International Conference on Bioinorganic Chemistry (ICBIC-6); 51, [1-2], 404 (1993).

THIENOTHIOPYRAN SULFONAMIDES - A NOVEL CLASS OF COMPLEXING AGENTS FOR THE PREPARATION OF DUAL CARBONIC ANHYDRASE INHIBITORS

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Although heterocyclic sulfonamides were only recently investigated as complexing agents,[1] it was proved that coordination compounds of widely used sulfonamide inhibitors of the enzyme carbonic anhydrase (CA, EC 4.2.1.1), such as acetazolamide,[2,3] methazolamide[4,5] and ethoxzolamide[2-5] with a range of main-group and transition metal ions, behave as very strong inhibitors of isozymes CA I and CA II. This is due to the mechanism of action of such inhibitors, which is dual, i.e., by sulfonamido anions, as well as cations.[6]

Here we report the first complexes of sulfonamides a and b, which are water soluble CA inhibitors, used in the treatment of glaucoma[7-9] by the topic route. The Zn(II), Co(II) and Cu(II) complexes of these sulfonamides were prepared and characterized by elemental analysis, IR and electronic spectroscopy, magnetic and conductimetric measurements. The stoichiometry of M^{2+}:RSO_2NH^- is of 1:2, and the donor system of the ligands comprises the endocyclic sulphur atom and the ionized sulfonamidic nitrogen. The new complexes behave as very strong CA II inhibitors, with IC_{50} values in the range of 10^{-10} M.
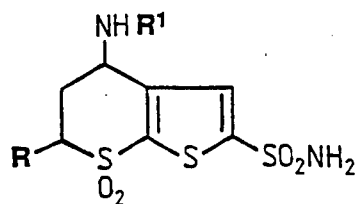
a   R = H; R^1 = Me$_2$CHCH$_2$-

b   R = Me; R^1 = Et

[1]G. Alzuet, S. Ferrer, J. Borras, C.T. Supuran, *Roum. Chem. Quart. Rev.*, in press.

[2]C.T. Supuran, M. Andruh, I. Puscas, *Rev. Roum. Chim.*, **1990**, 35, 393.

[3]C.T. Supuran, *Rev. Roum. Chim.*, **1992**, 37, 849.

[4]C.T. Supuran, G. Manole, M. Andruh, *J. Inorg. Biochem.*, **1993**, 49, 97.

[5]C.T. Supuran, *Roum. Chem. Quart. Rev.*, **1993**, 1, 77.

[6]C. Luca, M. Barboiu, C.T. Supuran, *Rev. Roum. Chim.*, **1991**, 36, 1169.

[7]a) G.S. Ponticello et al., *J. Med. Chem.*, **1987**, 30, 591; b) J.J. Baldwin et al., *J. Med. Chem.*, **1989**, 32, 2510; c) T.J. Blacklock et al., *J. Org. Chem.*, **1993**, 58, 1672.

[8]W.F. Brechue, T.H. Maren, *Exp. Eye Res.*, **1993**, 57, 67.

[9]E.A. Lippa et al., *Arch. Ophthalmol.*, **1992**, 110, 495.

CYANATE IS A SUBSTRATE OF CARBONIC ANHYDRASE

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Carbonic anhydrase (CA, EC 4.2.1.1), a zinc-containing enzyme, is a highly active catalyst for CO_2 hydration,[1] and was found to catalyze such reactions as aldehyde hydration,[2] or ester and sultone hydrolysis.[3,4] By substituting the Zn(II) ion with other cations, enzymes with different activities towards such substrates can be obtained.[5]

Recently, Supuran[6] proposed that cyanate, a powerful anionic CA inhibitor (K_I vs. CA II of 2×10^{-5} M),[7] might also behave as a substrate of this enzyme, being hydrolyzed to carbamate, which is highly unstable in solution and is spontaneously converted to NH_3 and CO_2. Since CO_2 is a CA substrate, in the end, the behavior of CNO^- is that of substrate as well as inhibitor. This might explain the controversy between crystallographic[8] and spectroscopic[9] data regarding the coordination of CNO^- to the metal ion. Computer simulations by Banci's group[10] showed that such a reaction pathway is possible.

Here we demonstrate that cyanate is indeed a substrate for CA II, CoCA II and CA III. Ammonia production was followed spectrophotometrically, in a cyanate solution with, and without the enzyme, in an assay based on the reductive amination of 2-oxoglutarate catalyzed by glutamate-dehydrogenase. In the presence of 19 μM of CoCA II, a 50 mM CNO^- solution lead to a catalytic production of 2 mM NH_3, in 11 days. The turnover number was 10^{-4} s^{-1}, compared to 10^6 s^{-1} for the CO_2 hydration reaction. This makes the two systems an excellent model for analyzing the role of binding energy in enzymatic catalysis. Thus, CO_2 which is loosely bound within the CA hydrophobic pocket is an excellent substrate, whereas CNO^-, being isoelectronic and isosteric with CO_2, but due to its negative charge, is bound very tightly. This makes it an inhibitor but also a substrate of this enzyme.

[1]T.H. Maren, *Physiol. Rev.*, **1967**, 47, 595.

[2]Y. Pocker, J.E. Meany, *Biochemistry*, **1965**, 4, 2535.

[3]R.E. Tashian, D.P. Douglas, Y.L. Yu, *Biochem. Biophys. Res. Commun.*, **1964**, 14, 256.

[4]E.T. Kaiser, K.W. Lo, *J. Am. Chem. Soc.*, **1969**, 91, 4912.

[5]I. Bertini, C. Luchinat, A. Scozzafava, *Struct. Bonding*, **1982**, 48, 45.

[6]C.T. Supuran, *Rev. Roum. Chim.*, **1992**, 37, 411.

[7]T.H. Maren, C.S. Rayburn, N.E. Liddell, *Science*, **1976**, 191, 469.

[8]M. Lindahl, L.A. Svensson, A. Liljas, *Proteins*, **1993**, 15, 177.

[9]I. Bertini, C. Luchinat, R. Pierattelli, A.J. Vila, *Inorg. Chem.*, **1992**, 31, 3975.

[10]Z. Peng, K.M. Merz, Jr., L. Banci, *Proteins*, **1993**, 17, 203.

Structure, Properties and Design of Type 1 (Blue) Copper Proteins

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The Cu(II) state of type 1 copper proteins is characterised by an intense blue colour, absorbance peak at ~600nm (ϵ 2000-6000$M^{-1}cm^{-1}$), and EPR small hyperfine coupling (A_{11} ~0.005cm^{-1}). The proteins are involved in electron transport, Cu(II) + e^- $\rightleftarrows$ Cu(I). Plastocyanin, which is a component of photosynthetic electron transport (PSII → PSI) in the chloroplast of higher plants and algae, has to date been the most extensively studied.

Properties of eight type 1 proteins are summarised in the Table below. Crystal structures are now available for the first five of these proteins.[1-4]

Protein	Source	Amino acids	pI	E^0 (mV)	λ_{max} (nm)	ϵ (M^{-1} cm)
Plastocyanin	Higher plants/green algae	99	4.2	375	597	4500
Azurin	Denitrifying bacteria	128	5.4	305	625	5200
Pseudoazurin	Denitrifying bacteria	123	7.65	—	593	2900
CBP	Cucumber	96	10.5	317	597	3400
Amicyanin	Methylotropic bacteria	106	4.7	260	596	3900
Rusticyanin	*Thiobacillus ferrooxidans* bacteria	144	9.1	680	597	2240
Stellacyanin	Lacquer tree	107	9.9	184	608	4080
Umecyanin	Horseradish roots	125	5.8	283	610	3400

The emphasis will be on recent results obtained from studies on the reactivities of pseudoazurin (*A. cycloclastes*), amicyanin (*T.versutus*), umicyanin (horse-radish roots), and rusticyanin (*T.ferrooxidans*). Three of the eight proteins exhibit reversible protonation and dissociation of the exposed active site His in the accessible range of pH. In the case of amicyanin the acid dissociation constant pK_a (6.7) for this process, is higher than that for plastocyanin (4.9), and pseudoazurin (4.7). There is a correlation with number of amino-acids between the His Cys Met residues coordinating the Cu. NMR and cross-reaction studies provide information regarding electron self-exchange rate constants. Results for line-broadening of ^{1}H peaks in the presence of redox inactive Cr(III) complexes and other evidence provides support for dual-site reactivity. Umecyanin and stellacyanin exhibit a colour change at pH ~9 corresponding to an isomerisation process, consistent with Gln instead of Met coordination. Thus the amide O-atom coordinates at low pH and the amide N-atom at high pH. An explanation of the high reduction potential (E°') for rusticyanin will also be considered.

1. A G Sykes, Adv.Inorg.Chem., 1991, 36, 377-408; Struct. Bonding, 1990, 75, 175-224.
2. E T Adman et al, J.Biol.Chem., 1989, 264, 87.
3. B A Fields, J M Guss and H C Freeman, J.Mol.Biol., 1991, 222, 1053.
4. A Romero, H Nar, A Messerschmidt, A P Kalverda, G W Canters, R Durley and F S Mathews, J.Mol.Biol., 1994, 236, 1196.

INTRAMOLECULAR d-π INTERACTION OF Co(II)- AND Ni(II) AZACROWN ETHER HAVING AN AROMATIC PENDANT MOIETY

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It has been well known that transition metal centered oxygenases, such as cytochrome P-450 and phenylalanin hydroxidase, exhibited the enzymatic action coupled with the certain reductase or co-enzymes. Metal center of these metalloenzyms, taking the resting state, have never been reduced by the reductases. The metal center of the activated form of the enzyme, in which substrate molecule bind at the reactive site, have been believed to be reduced by these reductases or co-enzymes.

We have studied metal-substrate d-π, of low-spin Ni(II)- and Co(II)-azacrown ether complexes, having aromatic pendant moiety, by means of X-ray-analysis, ESR, NMR and of electrochemical measurements. The results of X-ray analysis for the Ni(II) complex demonstrated that the aromatic moiety located at the axial position of the Ni(II) ion. The results of paramagnetic NMR measurements, carried out for the low-spin Co(II) complexes, justified the fact that the aromatic moiety also exist at the axial position of Co(II) ion, even in solution. Interestingly, the Co^{3+}/Co^{2+} formal reduction potentials of these Co(III) complexes, having the π-stacking structure, showed a positive shift about 150 mV. These observation trends suggest that the d-π interaction, between Co(III) and aromatic moiety, made the reduction potential of the complex positive shift .

References; 1)K. Tajima, K. Ishizu, N. Azuma, Inorg. Chem. Submitted for publication (1994)

MECHANISTIC INVESTIGATION ON REDUCTIVE DECOMPOSITION
OF Fe(II)OEP-OXYGEN COMPLEX TO BILIVERDIN SPECIES

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On the basis of the results obtained from ESR, optical absorption and electro-
chemical measurements, a possible mechanism of the reductive decomposition of
Fe(II)OEP-oxygen complex to biliverdine spcies will be discussed. ESR and optical
absorption spectra ascribable to be Fe(III)OEP-hydrogen peroxide complex (A;
$g1=2.28$, $g2=2.171$ and $g3=1.953$; 1 max. 562 and 604 nm)[2,3] was recorded for
the frozen DMF-pyridine (1:1) solution composed of Fe(III)OEPCl (1.0mM, 0.4
ml) and ascorbic acid (2M, 0.01ml). On standing the solution at -50 C for 1hr, the
hydrogen peroxide complex completely changed to Fe(II)octaethyl-verdo-heme (B),
which was isolated from the solution and identified based on the NMR and elements
analysis. The one-electron reduction of complex B gave the π-neutral radical
(denoted as complex C; g =2.000 and g =1.935) species.[4] The complex C was
assumed to be the intermediate species in the heme cleavage reaction, since, the
radical species readily changed to biliverdine by exposure to oxygen. In addition,
one-electron reduction of Zn(II)verdoheme also gave the π-neutral radical species[5]
and was also decomposed to biliverdine under oxygen condition. These observations
suggest that the central metal ion of the p-radical species do not take a part of the
cleavage reaction of verdoheme to biliverdine species.

References. 1)R. Lemberg, **Biochem. J.**, 29, 1322 (1935); 2)K. Tajima, **Inorg.
Chim. Acta**, 163, 115 (1989); 3)K. Tajima, M. Shigematsu, J. Jinno, K. Ishizu, H.
Ohya-Nishiguchi, **J. Chem. Soc., Chem. Commun.**, 144 (1990); 4) K. Tajima,
K. Tada, A. Yasui, K. Ishizu, H. Ohya-Nishiguchi, *ibid*, 282 (1993); 5) K. Tajima,
K. Ishizu, H. Ohya-Nishiguchi, **J. Bioinorg. Chem.**, 51, 283 (1993).

Studies on monomeric iron-sulfur centers and their association with other non-heme iron centers

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Monomeric iron-sulfur centers are composed of one iron atom connected to four cysteinyl residues. These types of centers were first identified in small electron carriers named Rubredoxins (Rd). They have been extensively studied by several spectroscopic techniques and their structure has already been solved. But, even in such a simple center, there can be variations. An example is the center present Desulforedoxin (Dx) from *D.gigas*. It is believed that this center presents the same type of coordination, yet a different type of rhombicity that implies also new spectroscopic properties. In an attempt to further characterize this center, it was possible to fully synthesise chemically the small Dx mimetic polypeptide chain (36 a.a.) and the center was then reconstituted with ^{57}Fe. The resulting syntetic protein has identical uv-visible, EPR and Mössbauer spectroscopic properties as the native protein.

These centers were also found in association with other non-heme type centers:

Rubrerithryn is a dimeric protein purified from *D.vulgaris* Hildenborough and *D.desulfuricans* ATCC 27774. It was shown that these proteins contain one Rd type center and one binuclear center that resembles the binuclear centers present in Ribonucleotide Reductase or Hemerithryn.

Desulfoferrodoxin is a monomeric protein that contains two iron atoms per molecule arranged as following: a Dx type center and a novel monomeric center with spectroscopic properties typical of penta or hexacoordination with S, N and/or O ligands.

Moura, I., Huynh, B.H., Hausinger, R.P., LeGall, J., Xavier, A. and Münck, E. (1980) *J. Biol. Chem.* **255**, 3242-3244.

LeGall, J., Prickril, B.C., Moura, I., Xavier, A.V., Moura, J.J.G. and Huynh, B.H. (1988) *Biochem.* **27**, 1636-1642.

Moura, I., Tavares, P., Moura, J.J.G., Ravi, N., Huynh, B.H., Liu, M.Y. and LeGall, J. (1990) *J. Biol. Chem.* **265**, 67-70.

Tavares, P., Ravi, N., Moura, J.J.G., LeGall, J., Huang, Y.-H., Crouse, B.R., Johnson, M.K., Huynh, B.H., and Moura, I. (1994) *J. Biol. Chem.*, in press

CRYSTALLIZATION AND PRELIMINARY X-RAY ANALYSIS OF A NEW CRYSTAL FORM OF NITRITE REDUCTASE FROM *Pseudomonas aeruginosa*.

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Nitrite reductase (NR) from *Pseudomonas aeruginosa* (EC 1.9.3.2), a redox enzyme synthesized by the bacterium grown in the presence of nitrate, is a soluble dimer of two identical subunits of 60 kDa, each one containing one c and one d_1 heme as prosthetic groups. The c heme is the electron accepting pole of the molecule and is reduced *in vivo* by either azurin or cytochrome c_{551} or both. The d_1 heme is a chromophore unique to the class of dissimilatory heme containing nitrite reductases, whose peculiar structure has been only recently delucidated and is the binding site for nitrite.

Crystals of *Ps. aeruginosa* NR have first been obtained by Yamanaka, and later by Takano and Akey, but proved unsuitable for high resolution X-ray diffraction studies. Very recently, single crystals of *Th. pantotropha* NR have been obtained by vapour diffusion in the presence of 2.2-2.3 M ammonium sulphate. These crystals belong to space group $P2_1$, with cell dimensions a=107.8 Å, b=61.9 Å, c=101.2 Å and β=112.5°; they diffract to 1.5 Å and are suitable for X-ray analysis. Nevertheless, the thorough functional characterization achieved in the case of *Ps. aeruginosa* NR as compared to *Th. pantotropha* enzyme, the primary structure of which has not yet been determined, the availability for the *Ps. aeruginosa* protein of an expression system and a specific NR defective *Ps. aeruginosa* strain, make *Ps. aeruginosa* NR particularly interesting and induced us to re-examine the crystallization problem.

A new crystal form of *Ps. aeruginosa* NR in the oxidized state, suitable for X-ray structure determination, has been obtained by vapor diffusion at 20°C, in the presence of PEG 4000 10%, TRIS/HCl 50 mM pH 8.35, NaCl 300 mM and at a protein concentration of 14 mg/ml. The crystals are dark green baguettes of dimensions 1.5 mm x 0.2 mm x 0.2 mm for the largest ones. These crystals are tetragonal, space group $P4_12_12$ or the enantiomorph, with cell dimensions a=b=127.7Å, c=172.7 Å. They diffract up to 2.9 Å on a MAR imaging plate/rotating anode. Assuming a dimer in the asymmetric unit, the Vm value is 2.93 Å^3/Da (58 % of solvent). Structure determination using heavy atoms derivatives is currently in progress.

Substrate Analogues and Site Specific Mutations Used to Understand the Mechanism of Nitrogenase

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Klebsiella pneumoniae nitrogenase comprises two metallo-proteins, the MoFe protein (Kp1; M_r 220,000, $\alpha_2\beta_2$, with 30 Fe and 2 Mo atoms) and the Fe protein (Kp2; M_r 67,000, γ_2, single 4Fe4S centre). The MoFe protein contains two types of metal clusters, the FeMo-cofactor (MoFe$_7$S$_9$, considered to be the site of substrate binding) and the "P"clusters (two pairs of bridged 4Fe4S -4Fe4S clusters). Both proteins are required for the reduction of N_2 to $2NH_3$ with concomitant evolution of H_2 and the hydrolysis of MgATP.

The recently published X-ray structures of the MoFe and Fe proteins (see Rees *et al.*, and Bolin *et al.*, 1993) will be briefly reviewed and used to help interpret recent spectroscopic and transient kinetic data on native and mutant forms of the enzyme. In particular, the mechanism of energy transduction involving the hydrolysis of MgATP, electron and proton transfers will be discussed and comparisons made with other ATP/GTP utilising systems such as actomyosin and p21ras (Thorneley 1992). The key energy transduction reaction in these systems is associated with the Pi release step. A high resolution, stopped-flow fluorimetric method, developed for the actomyosin S1 system (Brune *et al.* 1994), has been used to monitor Pi release from functioning nitrogenase. The kinetically complex time course has been simulated using our published model (Lowe & Thorneley 1984) with two new partial reactions in the Fe protein cycle, one of which is the Pi release step.

Brune, M., Hunter, J.L., Corrie, J.E.T. & Webb, M.R. (1994) Biochemistry, in press
Bolin, J. T. *et al.* (1993) in "ACS Symposium Series 535, Molybdenum Enzymes, Cofactors, and Model Systems", E. I. Steifel, D. Coucouvanis & W. E. Newton eds, pp.186-195.
Lowe, D. J. & Thorneley, R. N. F. (1984) Biochem. J. **224**, 877-886.
Rees D. C. *et al.* (1993) "ACS Symposium Series 535", see above, pp.170-185.
Thorneley, R. N. F. (1992) Phil. Trans. Royal Soc. London (B) **336**, 73-82.

Determination of the Functional Role of Subdomains of Human Extracellular Superoxide Dismutase by use of Protein Fusions

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During the past few years the involvement of oxygen free radicals has been observed in a number of degradative processes and diseases. However, as of yet, little is known about either the mechanisms of action of these radicals in the mentioned processes or how the defence against toxic oxygen radicals is organized.

Aerobic organisms have developed an array of defence mechanisms against oxygen free radicals, and superoxide dismutases (SODs) take part in this defence. Mammalian cells produce two different types of CuZn-SOD, an intracellular and one extracellular form (EC-SOD). The human extracellular SOD (hEC-SOD) forms a tetramer that is secreted, and it binds to proteoglycanes (especially heparansulfate) which are found on the surface of almost every cell in the human body.

The central part of the hEC-SOD sequence is clearly homologous with the sequence of the intracellular CuZnSODs, including the metal-binding ligands and SS-forming cysteines that have been demonstrated in CuZnSOD by X-ray crystallography. However, the 48 N-terminal amino acids of hEC-SOD have no counterparts in intracellular CuZnSODs; approximately 28 of the C-terminal amino acids in hEC-SOD have no counterparts in intracellular CuZnSODs. These hEC-SOD domains have been studied by use of fusion proteins comprised of the defined domains and human carbonic anhydrase II (HCAII). This technique allows EC-SOD domains to be studied separately; moreover, the fusion to HCAII allows a rapid and gentle one-step purification by affinity chromatography.

When the 49 N-terminal amino acids of hEC-SOD was fused to the N-terminal of HCAII (=FusNN) a well defined structure was formed and tetramerisation occured. Fusion of the 25 C-terminal amino acids of hEC-SOD to the C-terminal of HCAII (=FusCC) resulted in the formation of a monomer, which bound to heparin-Sepharose.

THE INFLUENCE OF NEW PLATINUM COMPLEXES ON THE SOME CELLULAR ENZYMES

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The main problem with the platinum drugs concerns with their strong toxicity as well as other side effects. It has been postulated [1] that a significant aspect of nephrotoxicity , bone marrow damage and gastrointestinal toxicity induced by platinum agents may involve ligand exchange reactions of complexes by sulfhydryl groups with subsequent inactivation of essential enzymes or other proteins.

A detailed investigation of the impact of platinum on three enzymes rich in thiol groups and engaged in substantial cellular metabolism was carried out. The activity and structure of malate dehydrogenase (E. C. 1.1.1.37, MDH), lactate dehydrogenase (E. C. 1.1.1.27, LDH) and pyruvate kinase (E. C. 2.7.1.40, PK) were studied under the influence of two new platinum agents:1/ cis-dichlorobis(4-vinylpyridine)platinum(II) and 2/ cis-dichlorobis(1-propylimidazole)platinum(II) as well as cisplatin and potassium tetrachloroplatinate. The compounds obtained by us possess considerable cytotoxic activity in vitro as was indicated earlier [2].

Our studies indicate that all these platinum complexes inhibit the enzyme activities at different levels of platinum concentration. Using CD spectroscopy the same systems were studied to assess the relationship between induced by platinum changes in enzyme activities and in the structure of enzymes. Platinum complexes evoked significant changes in the secondary structure of all three enzymes.

References

1. P. C. Dedon, F. R. Borch, Biochem. Pharmacol., 36 (1987), 1955.

2. J.Kuduk-Jaworska, Arch. Immunol. Ther. Exp., 42 (1994), 77.

Towards Structural Models for the Vanadium Centre in Vanadium Nitrogenase

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The vanadium centre in vanadium-nitrogenase of Azotobacter possibly carries the same ligand set as Mo in Mo-nitrogenase, *viz.* sulphur, nitrogen (His) and oxygen (homocitrate) donors. The oxidation state varies between +II and +III. We have started a programme to model the active site by using appropriate donor sets and vanadium mainly in medium oxidation states. Compounds **1** to **3** (see Figure) have been prepared according to eqns. (1) and (2) and characterized by, *inter alia*, X-ray diffraction spectrometry.

$$VOCl_2(thf)_2 + NaSN \longrightarrow VO(SN)_2 \quad \textbf{1} \qquad (1)$$
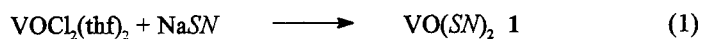
$$HSN = \text{2-mercaptopyridine-}N\text{-oxide}$$

$$VCl_2(tmeda)_2 + Na_2SS'S'S \begin{cases} \longrightarrow V(SS'S'S)(tmeda) \quad \textbf{2} \qquad (2a) \\ \\ \longrightarrow V_3(\mu\text{-}O)_2(SS)_4(tmeda)_2 \quad \textbf{3} \quad (2b) \end{cases}$$
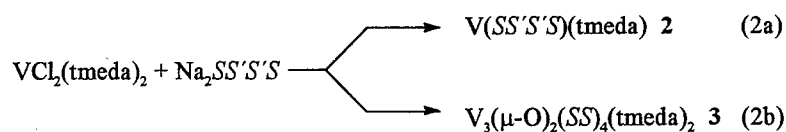
$H_2SS'S'S$ is a tetradentate sulphur ligand containing 2 thioether (*S'*) and 2 thiolate (*S*) donor functions. The formation of **3**, formally a $(V^V)_2(V^{II})$ complex, is accompanied by C-*S'*-bond cleavage, oxydation of the precursor compound and incorporation of O^{2-}.

1   **2**   **3**
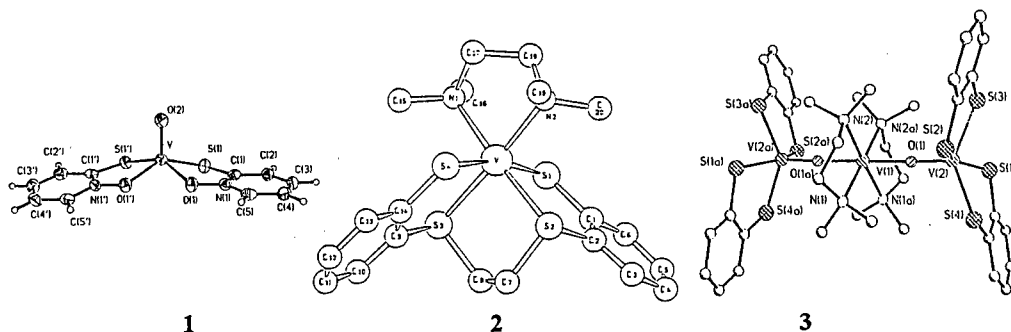
Reference: W. Tsagkalidis, D. Rodewald, D.Rehder and V. Vergopoulos,

Inorg. Chim. Acta, **219** (1994) 213-215.

Carbon-13 NMR Studies of Haem Electronic Structure

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The earliest NMR and epr studies of oxidised haem proteins revealed that the the unpaired electron distribution is highly asymmetric. This is manifested by three different g-values in epr and widely differing paramagnetic shifts for the haem substituents in NMR. These observations raised two questions: does the asymmetry have any biological significance, and what controls it? It has been speculated that the asymmetry is associated with the mechanism of electron transfer and that it is caused by a methionine axial ligand in His-Met cytochromes, or just one of the histidine ligands in His-His cytochromes, or by the haem substituents, or even by the surrounding protein. In short, these questions remained unanswered.

The basic theories of low spin iron epr spectra and of haem molecular orbitals are well established; the main difficulty has been the lack of experimental data. This is solved by measuring the Fermi contact shifts of carbon nuclei attached to the haem and we can now show that 1) the asymmetry is dominated by the orientation of *both* of the axial ligands, 2) that the rhombic distortion induced by a Met ligand is similar to that induced by His, and 3) that the energy differences are very small and probably have no biological significance.

This conclusion certainly does not mean that the asymmetry is of no interest since it can now become a source of precise structural information. The necessary data, g-values and NMR assignments for the haem substituents, are much more readily accessible than a full set of long-range NOE's. They then provide a direct measure of the orientation of the axial ligands together with a measure of the magnitude and orientation of the dipolar field which can be used as an aid for assignment and a tool for structure refinement.

NMR Studies of the Mechanisms of Cooperativity in Cytochrome c_3 from *Desulfovibrio* spp

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Cooperativity between different regions of a protein is extremely important in order to achieve some specific functions. *Desulfovibrio* spp cytochrome c_3 is a small (MW=13kDa) tetrahaem protein which exhibits cooperativity. NMR studies on this protein have shown that the four haems and acid/base group(s) constitute an interdependent consortium: the haem redox potentials are pH dependent (redox-Bohr effect) and each haem redox potential is dependent on the oxidation state of the other three haems (redox interaction potentials).

The thermodynamic parameters which govern the mechanisms of cooperativity in the cytochrome c_3 isolated from both *Desulfovibrio vulgaris* and *Desulfovibrio gigas* were determined, using the paramagnetic shifts of haem methyl groups in the NMR spectra of intermediate oxidised states at several pH's.

A model is suggested in order to explain how the network of positive and negative cooperativities between the four haems and acid/base group(s) allow the protein to undergo a $2e^-$ exchange with coupled H^+ transfer.

Ca^{2+} binding to chimeric proteins from lysozyme and α-lactalbumin

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Despite their homologous structure, c-type lysozymes and α-lactalbumins differ profoundly in their enzymatic functioning and in their unfolding behavior. Especially the latter property has been studied by several authors in that the α-lactalbumins enter a partially unfolded collapsed state (the molten globule) whereas lysozymes unfold cooperatively to a highly unfolded state. This different unfolding behavior seems to be related to the Ca^{2+}-binding property of these proteins. Whilst lysozymes are known to be unable to bind Ca^{2+} ions, the α-lactalbumins are binding Ca^{2+} strongly. Ca^{2+}-bound lactalbumin unfolds in a cooperative two-state process like lysozyme; dissociation of Ca^{2+} from α-lactalbumin, however, leads to the appearance of the molten globule state.

In order to understand the processes involved in the folding of the homologous proteins, lysozyme and lactalbumin, we constructed hybrid proteins in which a large part of the human lysozyme molecule is exchanged for the homologous counterpart of bovine α-lactalbumin. Two hybrids have been constructed and expressed in *S. cerevisiae* : Lyla1 in which the Ca^{2+} binding loop and the adjacent central helix from α-lactalbumin (residues 77-103) have been inserted into lysozyme and Lyla3 in which the terminal halve (residues 77-130) has been transplanted. Both hybrids have shown to be pure, compact and well structured proteins.

In the present contribution the binding of Ca^{2+} to both these chimers has been proven by microcalorimetric experiments and also by competition experiments with Fura-2. Thermal stability and unfolding behavior of the apo and the Ca^{2+} bound proteins have been analyzed by circular dichroism experiments and compared with the corresponding properties of the parent proteins.

GLUTAMATE SYNTHASE, A COMPLEX IRON-SULFUR FLAVOPROTEIN : EXPRESSION AND PROPERTIES OF THE ACTIVE αβ PROTOMER, AND OF THE ISOLATED α AND β SUBUNITS

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Glutamate synthase (GltS) is a complex iron-sulfur flavoprotein which catalyzes the reductive transfer of the amide group of glutamine to the C(2) carbon of 2-oxoglutarate.

The enzyme from *Azospirillum brasilense*, a Gram-negative nitrogen-fixing bacterium, is strictly specific for NADPH as the electron donor, is composed of two dissimilar subunits (α subunit ≈ 50 kDa; β subunit ≈ 160 kDa) and it contains 1 FAD, 1 FMN and several iron-sulfur centers per αβ protomer (≈200 kDa). A combination of kinetic and spectroscopic experiments have shown that during catalysis NADPH reduces the flavin at Site I; the electrons flow through the $[3Fe-4S]^{0,1+}$ center (FeS_I) and a second iron-sulfur center (FeS_{II}, presumably a $[4Fe-4S]^{1+,2+}$ center) to the flavin at Site 2, where reduction of the iminoacid takes place. A third iron-sulfur center, FeS_{III}, also presumably a $[4Fe-4S]^{1+,2+}$ center, is present. This center, which could only be reduced with the light/deazaflavin system, seems to have a potential too low to be involved in the intramolecular electron transfer process [1].

The genes encoding the α and β subunits of *Azospirillum* GltS have been recently cloned and sequenced, allowing us to deduce the amino acid sequences of GltS subunits, and to tentatively identify cofactors and substrate binding regions within the enzyme subunits [2, 3].

In order to obtain large quantities of *Azospirillum* GltS, which will allow us to characterize in greater detail the properties of the enzyme cofactors, flavins and iron-sulfur centers, the overexpression of the enzyme αβ protomer, and of the isolated α and β subunit was undertaken.

We were indeed able to overexpress the active αβ protomer as well as the isolated α and β subunits of *Azospirillum* GltS in *E. coli*, using pT7-7 based expression vectors and extensive engineering of the original 10 kb EcoRI fragment of *Azospirillum* DNA. Procedures for the isolation of the three protein species are being optimized in order to determine their properties. Experiments are in progress to further characterize the isolated α and β subunits of *Azospirillum* GltS, as well as the enzyme expressed in *E. coli*, with special emphasis on the kinetic properties of the proteins and on the properties of the bound flavin cofactors and Fe/S centers.

1. M.A. Vanoni, D.E. Edmondson, G. Zanetti and B. Curti *Biochemistry*, **31**, 4613 (1992)

2. R. Pelanda, M.A. Vanoni, M. Perego, L. Piubelli, A. Galizzi, B. Curti and G. Zanetti *J. Biol. Chem.*, **268**, 3099, (1993)

3. M.A. Vanoni, A. Mazzoni, P. Fumagalli, A. Negri, G. Zanetti and B. Curti *J.Biol. Chem.* (1994) submitted

ANTI-HERPES VIRUS EFFECT OF ZINC-COMPLEXES

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The effect of complexes of Zinc with picolinic acid /Zn(pic)$_2$/ and with asparaginic acid /Zn(asp)$_2$/ on the replication of HSV-1, HSV-2 and VZV was studied using multi-cycle and single-cycle growth tests, immuno-assays and electron microscope. As compared to VZV, a reversible inhibition of viral replication was only obtained when the higher nontoxic concentrations of both complexes are applied. However, both complexes expressed a therapeutic effect on HSV infection influencing free viruses, adsorption, immediately early and early steps of viral replication, as well as the expression of ICP4, ICP8 and glycoproteins from the envelope. Ferthermore, the amaunt of viral progeny with low electron density cores increased as compared to viral control. The prophylactic effect was manifested when the cells are pretreated with Zn-complexes. The data show that the mode of action of Zn(pic)$_2$ and Zn(asp)$_2$ on Herpes virus infection depends mainly of the viral specificity. The role of the ligand is also manifested. Thus, Zn(asp)$_2$ strongly inhibits the replication of HSV-1 and HSV-2 as compared to Zn(pic)$_2$. Zn(asp)$_2$ as OXIRICH is under development by Pharmacia Ld, Dupnitza, Bulgaria.

Equilibrium and spectroscopic studies on transition metal complexes of peptides containing bis-imidazole ligands

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Some bis-[imidazol-2-yl] derivatives containing the fragments of the specific sequence -Pro-Leu-Gly-Ile-Ala-Gly- cleaved by vertebrate collagenases at Gly-Ile bond were synthetised for the study of the specific inhibitors of zinc enzymes.

The copper(II) and zinc(II) complexes of these ligands and some other bis-[imidazol-2-yl]-methyl group containing agents were studied by potentiometric, VIS and EPR spectroscopic methods. The deprotonation of the imidazole groups occurs at lower pH-range, as compared to free imidazole, because of the electrostatic effect between the deprotonated imidazole N-atoms.

The data obtained for the copper(II) and zinc(II) complexes of the ligands revealed the formation of stable mono- and bis-complexes with two and four imidazole-N in coordination. In the case of ligands containing strongly-coordinating donor groups (carboxyl, amino, histidyl-imidazole) the complex formation processes are, however influenced by the side-chain residues. The axial coordination of the histidyl-imidazole residues is supported by the spectroscopic data.

The presence of the amino-group (bis-[imidazol-2-yl]methanamine) changes the coordination mode of the ligands. Formation of a dimeric species and the equatorial coordination of the amino group is suggested from the EPR data.

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51V NMR Differentiation Between Diastereomers of V^V Schiff-base Complexes Containing Amino Acids as Constituents

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Vanadium(V) complexes with a *N,O* ligand donor set are of interest in the context of their potential as structural models for the active site of vanadate dependent peroxides from marine brown algae. We have used Schiff-base ligands containing amino acids such as glycine, alanine and phenylalanine. The compounds are prepared by reacting vanadium sulphate, the amino acid and an accompanying aromatic aldehyde in an acetate buffer under anaerobic conditions (cf. Scheme). Addition of alcohol to the green/blue VO^{2+} Schiff base complex and aeration yields the red-brown VO^{3+} complexes with the coordination expanded by ligation of alcohol. The complexes are present as mixtures of the *endo* and *exo* form, corresponding to two diastereomeric pairs of enantiomers. Long exposure to the alcohol leads to partial decomposition and the formation of the vanadate triester $VO(OR)_3$. The ^{51}V NMR (see Figure) clearly indicates the presence of *endo-* and *exo-* complex plus the triester. With wet CH_2Cl_2, blue dimeric, oxo-bridged complexes are obtained (cf. Scheme).
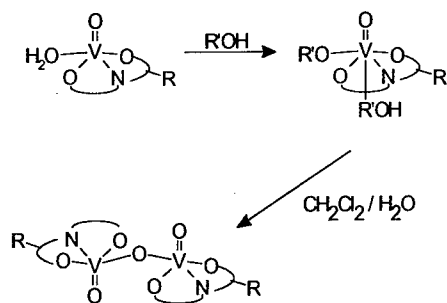
Scheme
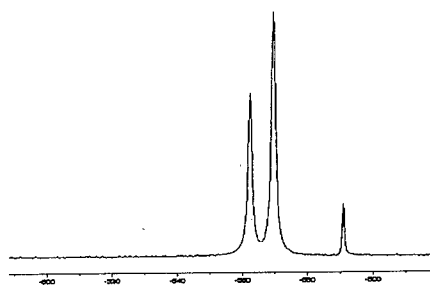
Figure

Reference

V. Vergopoulos, W. Priebsch, M. Fritzsche, D. Rehder. *Inorg. Chem.* **1993**, *32*, 1844.

New Non-oxo Vanadium-(IV) and -(V) Complexes with α-Hydroxycarboxylic- and Dicarboxylic Acids.

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Although the chemistry of oxovanadium-(IV) and -(V) complexes is well known, far less attention has been given to non-oxo (so called 'bare') complexes. We report here the preparation and characterisation of new non-oxo vanadium-(IV) and -(V) complexes with new N_2O_r- and NO_r-donor sets, constituing Schiff-base and α-hydroxycarboxylic- or dicarboxylic acids (cf. Scheme). Oxidation of the V^{IV} complexes with Ag^+ yields the corresponding non-oxo V^V complexes. The compounds were characterized by EPR, ^{51}V-NMR, CV and X-ray analysis (see Fig. for [V(salen)(benzilate)]).

$$VO(ONO) \quad + \quad SOCl_2 \quad \rightarrow \quad VCl_2(ONO)$$
$$VO(ONNO) \qquad\qquad\qquad\quad VCl_2(ONNO)$$

$$\downarrow + \text{Ligand} / - \text{LiCl}$$

<u>Scheme</u>

$$V(ONO)(Ligand)$$
$$V(ONNO)(Ligand)$$
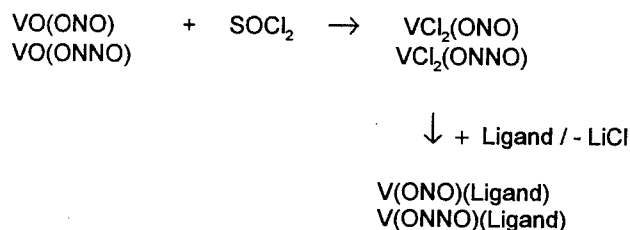
ONO : [N-(2-oxidonaphthalidene-phe-O)]²⁻   ONNO : (salen)²⁻
Ligand : Li₂-salt of dimethylmalonic-, benzilic-, 6-hydroxymethylpicolin, and salicylic acid.
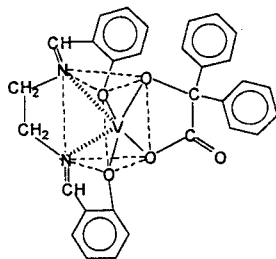
<u>Figure</u>

Reference

V. Vergopoulos, S. Jantzen, N. Julien, E. Rose, D. Rehder; *Z. Naturforsch.*, submitted

INTERACTIONS OF Pd(II)-HISTIDINE COMPLEXES WITH DINUCLEOTIDES

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The mechanism of cisplatin and other transition metal complexes binding to DNA has been widely studied in the last few years. It has been interpreted as the formation of bidentated chelates with two N atoms from neighbouring guanine bases[1]. Reedijk and col.[2] investigated the reaction of new antitumoral complexes of Pt(II), cis-[Pt(NH3)2(4-methylpyridine)Cl]Cl with d(GpG), and they report the preference for platinum binding at the 5'G in the dinucleotide. In other papers[3], the same authors have studied the conformation of the Pt(en)-dinucleotides in relation to the Pt(NH3)2-dinucleotides by ^{1}H NMR and CD spectroscopy, and they found only small differences in the spectra, suggesting the same structures for both adducts. Similar studies with other Pt(II)-complexes and oligonucleotides have been carried out[4]. We have also studied the reactions between Pt(II)-methionine complexes and d(ApA), d(ApG) and d(GpG) nucleotides[5] but in no case has the interaction of oligonucleotides with Pd(II) complexes been described.

Therefore the aim of our work was to study the nucleotide coordination sites as well as the possibility of chelate formation between the [Pd(D-Histidine)Cl2], [Pd(L-Histidine)Cl2], [Pd(L-HistidineOme)Cl2] and d(ApG) and d(ApA) dinucleotides. All Pd(II)-Histidine complexes were studied crystallographically and spectroscopically.

The reaction between the complexes and the dinucleotides was carried out in D_2O medium (monitoring the pH) in a NMR tube and studied by ^{1}H NMR spectroscopy. The H(8) signals corresponding to the guanine ring in the d(ApG) dinucleotide shifted significantly towards upper fields while the H(8) signals corresponding to the adenine ring in both d(ApA) and d(ApG) remained unshifted. The H(2) signal assigned to the Ap adenine in d(ApG) shifted towards upper fields. This observation may suggest that Pd(II) coordinates forming chelates d(ApG)-N3(1)-N7(2) and d(ApA)-N3(1)-N7(2). The conformation of the Pd(histidine)-dinucleotides is studied with CD spectroscopy and compared in both D and L isomers as well as in esters.

References

1. B.K.Kepler (ed.) *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, 1993.

2. E.M.L.Lempers, M.J.Bloemink, J.Brouwer, Y.Kidani and J.Reedijk; *J.Inorg.Biochem.*,**40**, 23 (1990)

3. C.J.van Garderen, M.J.Bloemink, E.Richardson and J.Reedijk; *J.Inorg.Biochem.*, **42**, 199 (1991)

4. N.Boogaard, C.Altona and J. Reedijk; *J.Inorg. Biochem.*, **49**, 129 (1993).

5. A. Caubet, V.Moreno, unpublished results.

NMR Study of the Metal Site in Metal-Substituted Stellacyanin

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Stellacyanin (St) from *Rhus vernicifera* is considered an outlier in the family of blue copper proteins (BCP's) due to its low redox potential (180 mV) and its puzzling spectroscopic properties. The most common copper ligands found in BCP's are two His, one Cys and a Met residue. A Gly may be found in some cases as a fifth ligand at a longer distance. St is lacking a methionine in its sequence, and the identity of the fourth ligand replacing Met has not been elucidated. Molecular modeling and sequence alignment studied led Fields *et al.* to the proposal that Gln97 may be the elusive ligand.[1] This is consistent with spectroscopic studies on the M121Q mutant of Azurin.[2]

Metal substitution has proved to be a helpful tool for probing metal sites. In particular, when Co(II) and Ni(II) are introduced, the NMR signals of nuclei in the neighbouring of the metal center experience large shifts. The ^{1}H NMR spectrum of Co(II)St has been already reported,[3] but some results are conflicting with those derived from molecular modeling. The present work was carried out in an attempt to assign the paramagnetically shifted signals and to shed some light regarding the metal site in stellacyanin.

A set of proton resonances isotropically shifted have been detected in Co(II)- and Ni(II)-substituted Stellacyanin which correspond to the metal-bound residues. The results are consistent with the metal adopting a distorted tetrahedral geometry. The proton signals corresponding to the two bound His and to the Cys residue have been identified and inequivocally assigned. It has also been shown that the metal site in St is more exposed than that of azurin. Since two His residues are solvent-exposed, it may be similar to that of the Cucumber Basic Protein. These two His residues are shown to be bound to the metal ion by its Nδ1 atom. The signals corresponding to the fourth ligand present dipolar connectivities which indicate that a Gln residue is bound to the metal ion.

1. Fields,B.A., Guss,J.M.; Freeman,H.C. *J.Mol.Biol.* **222**, 1053 (1991).
2. Romero,A.; Hoitink,C.; Nar,H.; Huber,R.; Messerschmidt,A.; Canters,G.W. *J. Mol.Biol.* **229**, 1007 (1993).
3. Dahlin,S.; Reinhammar,B, Ångstrom,J. *Biochemistry* **28**, 7224 (1989).

Zinc(II)-Thiolate and Zinc(II)-Sulfide-Thiolate Compounds

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In recent years, numerous metalloproteins have been found which contain $Zn(Cys)_4$ cores.

Zinc-containing metallothioneins (MT), for example, are low molecular weight proteins consisting of 61 amino acid residues, 20 of which are cysteine and bind seven to 12 heavy metal ions per polypeptide. The structure of Zn_2,Cd_5-MT consists of two domains, one containing a Cd_4S_{11} cluster, the other containing a $CdZn_2S_9$ cluster[1]. Furthermore, a large number of DNA-binding proteins have been found to contain zinc fingers with the general formula $Zn(Cys)_{4-n}(His)_n$, where n = 0-2. Human TFIIS, for example, contains a novel Cys_4 sequence which presumably coordinates tetrahedrally to a Zn^{2+} ion.[2]

Utilizing α,α'-o-xylenedithiole (H_2S_2-o-xyl) as a ligand we synthesized the metal-sulfide-thiolate compound $[BzMe_3N]_4[Zn_{10}S_4(S_2$-o-xyl)$_8]\cdot16$ MeOH. Figure 1 shows the anion which is the first tetraadamantanoid zinc cluster with a chelating dithiolate ligand. Like in MT the metal ions show distorted tetrahedral coordination geometry.

The reaction of $\alpha,2$-toluenedithiole with $ZnCl_2$ in methanol yields the mononuclear compound $[Ph_4P]_2[Zn(S-C_6H_4-CH_2-S)_2]\cdot MeOH$. It shows a coordination geometry which is similar to the one observed in Cys_4 zinc fingers.

We also use thiolate ligands with incorporated N-donors such as pyridine to synthesize zinc complexes with mixed S,N-coordination.

Figure 1: $[Zn_{10}S_4(S_2$-o-xyl)$_8]^{4-}$

[1] A. H. Robbins, D. E. McRee, M. Williamson, S. A. Collett, N. H. Xuong, W. F. Fury, B. C. Wang, C. D. Stout, J. Mol. Biol. **221**, 1269 (1991)

[2] X. Qian, S. N. Gozani, H. Yoon, C. J. Jeon, K. Agarwal, M. A. Weiss, Biochemistry **32**, 9944 (1993)

Synthesis and structure of new antitumour metal compounds.

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An important number of ruthenium complexes have been recently tested as promising antitumoral drugs. As has been claimed, the unique chemistry of ruthenium might be the key for future developing of a new generation of anticancer ruthenium compounds. As it is well known, DNA synthesis inhibition occur via a redox mechanism in which a cellular component reduces the Ru(III) to Ru(II) prior to binding; moreover, due to the hypoxic conditions often holding in tumor cells, the dipositive oxidation state is more likely to occur. For these reasons, Ru(III) complexes are considered as "predrugs" and behave as relatively nontoxic until activated by reduction.

In this work, new coordination compounds between ruthenium and chelating sequestering ligands have been sinthesized and characterized. RuCl$_3$ was used as starting material and the ligands were added in solid form. The resulting mixtures were heated following a variation of the well known hydrotermal synthesis and, after slow concentration of the obtained solutions, coloured crystals suitable for X-ray analysis were recovered from some of the preparations. The isolated complexes were tested for antineoplastic action and toxicity, in vitro and in vivo, against a wide variety of experimental tumors. Important activity was detected in most of the cases while the absence of toxicity was the most important finding.

All complexes present labile chloride ions bound to the metal in *cis* positions. Magnetic study, electronic spectroscopy and XPS study, indicate oxidation states of +3 and +4 for the obtained ruthenium complexes. Both oxidation states are considered as "source" of the reduced state +2, shown by the metal ion when located in the tumor hypoxic area.

In order to study some substitution reactions, several aquocomplexes were identified and isolated. Additional reactions of the ruthenium complexes with mono- and bidentate ligands were also performed; replacement of the labile monodentate ligands in *cis* positions were found in all cases. Some other reactions with nitrogenated compounds leading to the succesful fixation of dinitrogen were accomplished.

DNA Interactions of Antitumor Platinum(IV) Complexes

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In the search for new platinum drugs, a number of analogues of cisplatin containing platinum(IV) metal centers have been synthesized and evaluated in a variety of preclinical tumor screens. Numerous studies of the molecular mechanism of antitumor effect of platinum drugs have revealed that the antitumor activity stems from the interaction of these compounds with intracellular DNA. In order to address the direct binding mode of the platinum(IV) drugs, as well as other fundamental questions related to their mechanism of action, the studies described in this contribution were carried out.

Modifications of natural DNA and synthetic double-stranded oligodeoxyribonucleotides by *cis*-diamminedichloro-*trans*-dihydroxyplatinum(IV) (oxoplatin) were studied by means of immunochemical methods, Maxam-Gilbert footprinting techniques, high-pressure-liquid chromatography of enzymatically digested DNA, and transcription mapping. It was found that the platinum(IV) complex can bind DNA without prior reduction to the platinum(II) analogue. In addition, this interaction per se does not result in the reduction of the platinum(IV) center. The direct irreversible binding of the platinum(IV) drug is, however, slow as compared to the reaction of its platinum(II) analogue. Oxoplatin preferentially binds to guanine residues and forms DNA intrastrand and interstrand cross-links containing the platinum(IV) moiety. These lesions inhibit *in vitro* transcription by a prokaryotic DNA-dependent RNA polymerase. It has been suggested that the DNA adduct profile, and most likely also the mechanism of antitumor activity resulting from the direct binding of platinum(IV) complexes to DNA is similar to that of their platinum(II) derivatives. For these reasons the platinum(IV) complexes are also not assumed to eliminate difficulties that stem from inherent or acquired resistance to their platinum(II) analogues.

Models for the Active Sites of Zinc Containing Phosphatases

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Enzymes involved in phosphate transfer, e. g. from phosphate esters or oligophosphates, constitute an important class of zinc containing enzymes. Just like in other hydrolytic zinc enzymes it is likely that a Zn-OH unit is the active and working unit of the catalytic center. We have been modelling this unit by hydrotris(pyrazolylborato)zinc-hydroxide complexes. Specifically the pyrazolylborate ligand with 3-cumyl and 5-methyl substituents was found to be a potent nucleophile for the cleavage of diphosphates and phosphate esters.

This presentation deals with an extension of this work to more „natural" phosphate esters and to a new pyrazolylborate ligand. Using the cumyl substituted pyrazolylborate, p-nitrophenyl esters of ribose phosphate and of nucleotides could be cleaved by the L·Zn-OH complex. Formula **1** depicts one cleavage product.

1   **2**
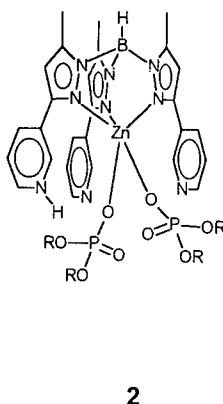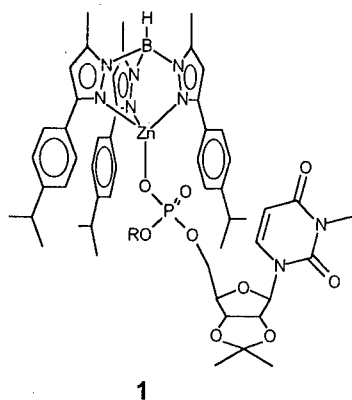
Using a pyrazolylborate ligand with 3-pyridyl substituents in the 3-positions, a more hydrophilic environment around the zinc ion is created. This has caused different reaction pathways and different modes of phosphate attachment to the metal. Formula **2** (R = p-nitrophenyl again) shows a reaction product which can be considered as an intermediate of diphosphate cleavage with both fragments being activated by zinc. The relevance of these findings for the enzyme processes will be discussed.

Planar and non-planar porphyrins and their oxoferryl π-radical cation complexes.

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Characterization of oxoferryl porphyrin π-cation radicals remains a challenge in the context of understanding and mimicking the oxidation processes catalyzed by various heme containing enzymes. These include horseradish-(HRP) lignin-(LiP) and chloro-(CPO) peroxidases and catalases (CAT) which catalyze the decomposition of hydrogen peroxide via an intermediate termed compound-I. The oxidation state of this intermediate (Cpd-I) lies two equivalents above the native ferric states of these enzymes: one of the oxidation equivalents is stored as a low-spin oxoferryl moiety ($|FeIV=O|$; S = 1) and the other is stored as a porphyrin-centered π-cation radical (S' = 1/2). HRP-I, CPO-I and CAT-I give unusual EPR signals that correspond approximately to one unpaired electron per heme group. It has been shown that these signals are a manifestation of an exchange interaction $H_{ex.} = JS \bullet S'$ between the ferryl iron (S =1) and the porphyrin radical (S' = 1/2), where J is the exchange parameter. Several synthetic oxoferryl porphyrin π-cation radical model systems have been obtained which can be analyzed similarly. For these compounds-I and their model systems, the spin coupling can be characterized by the sign of the exchange coupling parameter J, its relative magnitude and the zero-field splitting parameter D. Whereas in the model compounds obtained with planar tetraarylporphyrins (H_2TMP, H_2TDCPP, H_2TTMPP) the coupling is ferromagnetic and moderately strong (J>0; $|J|/D \geq 1$), in the compounds-I the couplings are either antiferromagnetic and extremely weak (HRP-I : J<0; $|J|/D < 0.1$) or antiferromagnetic and moderately strong (CPO-I: J<0; $|J|/D = 1.02$) or ferromagnetic with an intermediate strength (CAT-I: J >0; $|J|/D = 0.4$).

In general the observed exchange coupling is the result of a competition between two opposing tendencies and can be written as $J = J_F + J_{AF}$. The variation between ferro- and antiferro-magnetic coupling in the compounds-I and their model systems must reflect an increasing departure from idealized 4-fold symmetry. In such a symmetry, the half filled orbitals of $|Fe=O|^{2+}$ and the π molecular orbitals of the porphyrin radical moiety are orthogonal, leading to $J_{AF} = 0$ and $J = J_F >0$. The reduction of symmetry could be related to non-planar porphyrin cores and/or the π-bonding between the proximal axial ligand and the iron IV center.

We have now synthesized several β-pyrrole halogenated and arylated porphyrins, some of which present non-planar cores. Compound-I analogs containing these porphyrins have been prepared and characterized and oxoferrylporphyrin π-cation radicals have been obtained with different proximal axial ligands of iron(IV). The results of these studies will be presented and discussed.

Synthetic and Biologically Occurring Superoxide Dismutase Mimics

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Cu(II) Pu Phe Py [((N,N'-bis(2-pyridyl-phenyl)-methylene)-1,4-diaminobutane)-
(N,N',N'',N'''))-copper(II)] a copper(II) di-Schiff-base turned out to be in closest agree-
ment in both structure and catalytic function with the active centre of intact
Cu_2Zn_2SOD. In addition to the crystal structure the EPR parameters were $g_\perp = 2.05$ and
$g_\parallel = 2.217$, ε_{690} was 136 $M^{-1}cm^{-1}$. The pulse radiolytically determined superoxide dis-
mutation was successful even in the presence of a four fold molar excess of EDTA at a
rate of $0.5 \times 10^9 M^{-1}s^{-1}$. Furthermore, this complex survived competitive biochelation in
the presence of 0.7M serum albumin.

Ubiquitously occurring hexanuclear $Cu(I)_6$-thionein reacted with pulse radiolytically
generated $\cdot O_2^-$ and $\cdot OH$ without any detectable cleavage of the Cu(I)-thiolate bonding.
The intactness was controlled with both luminiscence and circular dichroism measure-
ments. The active component involved in the reversible oxidation of the oligonuclear
Cu(I)-thiolate centre was suggested to be a Cu(I)-stabilised thiyl radical. In a separate
EPR study employing yeast Cu(I)-thionein, GSH and Cu-GSH it was shown that indeed
thiyl radicals could be successfully generated from thiolate sulphur via oxidation using
both Ce(IV)-salts or photochemically generated superoxide at 77K. The g-value was
2.036. Apart from its efficient control of copper transport it is suggested that Cu(I)-
thionein is actively involved in the scavenging of oxygen free radicals by a reversible
thiolate oxidation reduction cycle. The transiently observed thiyl radical appears to be the
electron transferring site.

THE EFFECTS OF A CHARGED AMINO ACID RESIDUE ON THE OPTICAL AND CATALYTIC PROPERTIES OF MYELOPEROXIDASE.

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Myeloperoxidase is present in high concentration in polymorphonuclear neutrophils. The enzyme plays a role in the anti-microbial activity of these cells, since it catalyzes the formation of hypochlorous acid, which is a bactericidal agent. The prosthetic group of the enzyme is a heme group, the chemical nature of which is not identified, due to the fact that it is not possible to extract the heme from the protein. This is probably the result of a covalent linkage of the heme to the protein. NMR studies and studies on photochemically modified myeloperoxidase have indicated that the prosthetic group is similar to that of lactoperoxidase, which is an iron-protoporphyrin IX. Pyridine hemochrome spectra of myeloperoxidase are similar to those of heme *a*, which indicates the presence of a formyl group on the porphyrin. Also MCD studies on heme-substituted myoglobin are in favour of a formyl-substituted heme as the prosthetic group of myeloperoxidase. However, both MCD and resonance Raman studies have suggested an iron-chlorin as the prosthetic group of myeloperoxidase.

The optical absorbance spectra of myeloperoxidase are very peculiar. The spectrum of the reduced enzyme displays a Soret band at 472 nm and an α band at 636 nm, both of which are red-shifted as compared to other hemoproteins. It has not yet been possible to explain all the spectroscopic properties of the enzyme, but it has been suggested by us that a negatively charged residue is present close to the chromophore that interacts with the heme group and imposes the red-shift in the optical spectrum. The X-ray crystal structure of canine myeloperoxidase has been determined to 3 Å resolution and the data indicate that negatively charged carboxylate groups (Glu242 and Asp94) are indeed present close to the chromophore. It is possible to express the cDNA of human myeloperoxidase in Chinese hamster ovary cell lines and a 84 kDa monomeric single chain heme-containing enzyme is secreted in the medium with physiochemical properties identical to those of the mature enzyme.

We have used the site-directed mutagenesis technique to replace Glu242 by Gln and thus specifically removing the negative charge. The results show that the charged group in the vicinity of the heme macrocycle plays a pivotal role. The group affects the Soret peak postion and also induces the resonance Raman spectrum typical for an asymmetric porphyrin. The mutation also abolishes the chlorinating activity of the peroxidase. We conclude that the prosthetic group of myeloperoxidase is not an iron-chlorin but resembles heme *a*.

KINETIC AND SPECTROSCOPIC STUDIES OF

IRON STORAGE IN HORSE SPLEEN FERRITIN

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Most living organisms require iron for growth and bacteria are no exception to this rule. The body has developed an elegant array of proteins to carefully transport and store iron in order to prevent the accumulation of toxic amounts of free iron.

Ferritin is the iron storage protein, it consists of a soluble protein shell of 24 identical subunits. The sub-units are arranged to form a roughly spherical internal cavity within which a ferrihydrite core of variable iron content is deposited. Apoferritin catalyses the oxidation of ferrous iron to the ferric state. A variation in the copper content of apo (iron-free) and native ferritins has been reported (Bolann and Ulvik, 1993). Studies on horse spleen apo-ferritin and copper treated apo-ferritin show the initial rate of iron uptake and oxidation is observed to be greater in the copper treated sample. These results suggest a mechanistic link between the catalytic function of copper and iron uptake and oxidation.

Reference

Bjorn J. Bolann and Rune J. Ulvik, *F.E.B.S*, **328**, 263-267 (1993)

SPECIATION HEAVY METALS IN SOME BIOLOGICAL SPECIMENS.

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Speciation of heavy metal in investigated biological specimens were made by Rudd's method and it were determinated following forms: exchangeable form, absorptive, organic connection, carbonate, sulfide, soluble form. Successively obtained filtrates were examined for 7 heavy metals content: Pb, Cd, Cu, Ni, Mn, Zn, Fe by means of ASA (Varian Techtron 5DA).

In the test area figures for total precipitation of the remaining metals were as follows: 26 mgCu/m^{2}year, 10.2 mgCr/m^{2}year, 17.1 mgNi/m^{2}year, 381 mgMn/m^{2}year, 2081 mgZn/m^{2}year. The toxicological condition described results given in under Table:

Heavy metals contents in excrement of *Gallinula chloropus* and in its habitant.

Sample	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
bottom sediment [µg/g]	47.6	1229.0	18.7	47.1	115.7	3061.0	11.1	266.0
excrements [µg/g]	20.5	1117.0	1.3	7.7	11.8	1036.0	1.3	37.1
foods [µg/g]	8.4	498	1.3	2.2	8.3	647.0	1.4	33.0
air [ng/m^3]	0.012	0.200	0.020	0.010	0.300	0.700	0.009	0.400
water [µg/dm^3]	0.027	0.014	0.018	0.066	0.055	0.016	0.016	0.062

The enrichment factors of some heavy metals in comparison to given environmental compounds.

Source	Cr	Mn	Co	Ni	Cu	Zn	Cd	Pb
bottom sediment	0.4	0.9	0.1	0.2	0.1	0.3	0.1	0.1
foods	2.4	2.2	1.0	3.5	1.4	1.6	1.01	1.1
air (•10^4)	172	558	6.6	79.7	3.9	148	14.7	9.3
water	758	79700	73	117	214	66800	86	599

The heavy metals content in all analyzed compounds of the wintering moor hen's habitant had values characteristic for strongly contaminated sites. High content soluble forms of Pb, Zn in the excrement vegetation and in the abiotic environment is evidence, contamination of the given habitant and the moor hen's organisms with these elements. Metals, in all forms, and Mn, Cr, Mg were particularly intensively excreted by the moor hen's organisms.

Hemerythrin and Catalase Functional
Model Chemistry

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We have synthesized a series of diiron(III), dimanganese(III) model complexes and have investigated their reactivity toward hydrogenperoxide. The diiron(III) complex catalyzes the disproportionation of H_2O_2 provided that at least one of the ferric ions has a labile coordination site (Fe-OH_2). The mechanism of this reaction has been studied in detail. It represents reverse hemerythrin chemistry (dioxygen uptake and transport).

$$[\{LFe^{III}(\mu\text{-}O)(\mu\text{-succinato})Mn^{III}(bpy)(H_2O)\}_2]^{4+}$$
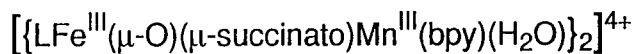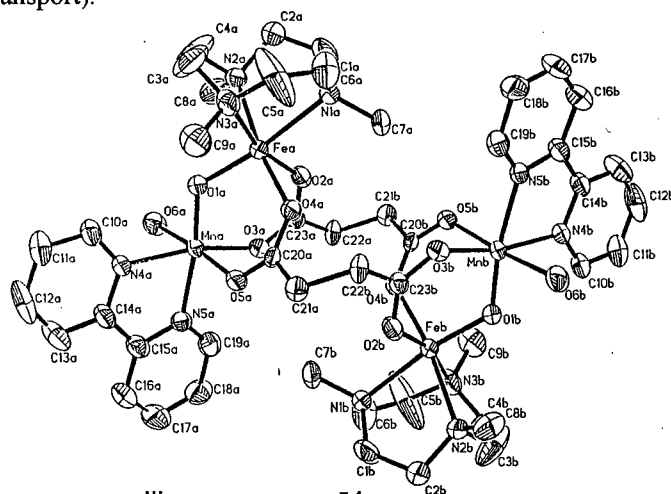
Di- and tetranuclear complexes of manganese(III) and their heteronuclear iron(III)/manganese(III) analogs have also been synthesized and their catalase activity has been established. Interestingly a mixed-valence di-μ-oxo-dimanganese(III/IV) species shows catalase activity. This form has been shown to be inactive in the chemically modified catalase isolated from Lactobacillus Plantarum. We will briefly review the possible mechanisms of H_2O_2 disproportionation of catalase models currently under investigation in our and other laboratories: more than one mechanistic pathway apparently emerge from these studies.

Catalysis of O$_2$ reduction and H$^+$ translocation by the respiratory oxidases.

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The respiratory oxidases form a family of structurally and functionally related enzymes that are responsible for the activation and reduction of O$_2$ in cell respiration. A binuclear haem iron/copper (Fe$_{a3}$/Cu$_B$) binuclear centre and a second low spin haem (Fe$_a$) are conserved features in the largest subunit of these enzymes, which are located in the inner mitochondrial membrane of eukaryotic cells or in the plasma membrane of bacteria. The electron donor may be either cytochrome c, or a quinol, in different enzyme variants. These enzymes conserve energy as an electrochemical proton gradient by linking the redox process to vectorial translocation of protons across the membrane. Time-resolved optical spectroscopy has shown that the earliest event in the O$_2$ reaction is the fast formation (8 µs at 1 mM O$_2$) of an equilibrium of states consisting, in sequence, of unbound reduced Fe$_{a3}$/Cu$_B$, Fe$_{a3}$/Cu$_B$-O$_2$ and Fe$_{a3}$-O$_2$/Cu$_B$ intermediates, as well as an activated form of the latter which can accept an electron from Fe$_a$. The O$_2$ is then trapped to the binuclear site site by eT from Fe$_a$ about 16 Å (Fe-Fe distance) away from Fe$_{a3}$ (32 µs), probably yielding a primary peroxy intermediate, so far only observed by optical spectroscopy (M. Verkhovsky et al. [1994] Biochemistry 33, 3079-3086). The proton translocation events are intimately coupled to those reactions of the catalytic cycle that carry out the oxygen chemistry (M. Wikström [1989] Nature, 338, 776-778). A hypothetical mechanism of proton translocation (the "histidine cycle") will be described, which involves a conserved histidine residue that cycles between imidazolate and imidazolium states with uptake and release of two protons, and intermittently becoming a ligand to Cu$_B$ (for details, see M. Wikström et al. [1994] Biochim. Biophys. Acta, in press).

CHARACTERIZATION OF THE NOVEL CYCLIC
HIGH-SPIN IRON(III) PORPHYRIN TRIMER

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The oligomerization process of a monomeric iron(III) 2-hydroxy-5,10,15,20-tetraphenylporphyrin complex (2-OH-TPP)Fe^{III}Cl has been investigated. We have determined the formation of the unprecedented cyclic trimeric complex [(2-O-TPP)Fe^{III}]$_3$. The spectroscopic evidences indicate that this trimer has a head-to-tail cyclic structure with the pyrrolic-alkoxide groups forming bridges from one macrocycle to the iron(III) ion in the adjacent macrocycle.
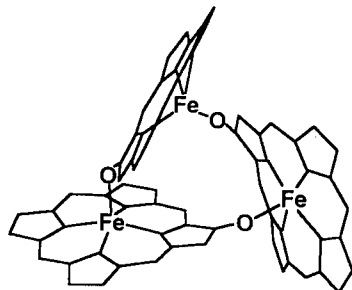
The presence of the three paramagnetic, weakly coupled high-spin iron(III) centers produces marked variation of positions and line widths for the pyrrole resonances in the ^{1}H NMR spectrum of the trimer. [(2-O-TPP)Fe^{III}]$_3$ is cleaved by protic acids (HX) to form high-spin, five-coordinate species (2-OH-TPP)Fe^{III}X. Addition of an excess of base (methoxide ion, hydroxide ion in methanolic solutions) converts [(2-O-TPP)Fe^{III}]$_3$ to the five-coordinate, high-spin complex [(2-O-TPP)Fe^{III}X]$^-$ (X = CH_3O^-, OH^-). The base-catalyzed exchange of the proton at the position next to the hydroxy group (3-H) has been observed for [(2-O-TPP)Fe^{III}X]$^-$ complexes and has been accounted for by the keto-enol tautomerism. The characteristic shifts of the 3-H resonances in [(2-O-TPP)Fe^{III}(OH)]$^-$, (2-OH-TPP)Fe^{III}Br, and (2-benzoyloxy-TPP)Fe^{III}Cl provided the direct insight into the electronic structure of tautomeric forms.

ELECTRON TRANSFER CHAINS IN *DESULFOVIBRIO GIGAS*: OLD AND NEW METALLOPROTEINS AND THEIR MECHANISMS

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Bacteria belonging to the genus *Desulfovibrio* are non-photosynthetic organisms that in strict anaerobic conditions perform the reduction of sulfate to sulfide in the presence of molecular hydrogen or low molecular mass organic compounds such as lactate, pyruvate, malate or aldehydes. Some of these bacteria, such as *D. gigas* can store energy by accumulating large amounts of polyglucose.

The diversity of substrates which can be used by these bacteria is only possible due to the presence of a complex network of electron transfer chains, involving a large variety of metalloproteins. The metals involved include Fe, Mo, Ni and Ca, in a wide number of architectures with different chemistry, stereochemistry and cluster arrangements. Several of the metalloproteins isolated from D. gigas are novel ones and their characterisation, functional, mechanistic and structural, had an important impact in Bioinorganic Chemistry research.

The recent characterisation of some previously known and some newly found centres as well as their functional utilization will be discussed.

Metal Complex–Tetrahydropterin Systems as
Phenylalanine Hydroxylase Models

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Phenylalanine hydroxylase (PAH) converts phenylalanine to tyrosine by activating O_2 in the presence of iron or copper and a pterin cofactor, 5,6,7,8-tetrahydrobiopterin. The active site of Cu-containing PAH has been concluded to have two coordinated imidazole nitrogens,[1] but the mode of biopterin–Fe or –Cu interactions and the mechanism of O_2 activation are largely unknown.

Reactions of 6,7-dimethyl-5,6,7,8-tetrahydropterin (H4DMP) with $Cu(NO_3)_2$[2] and $Fe(ClO_4)_3$ gave a protonated trihydropterin radical as the main species, showing that one-electron redox reactions occurred between H4DMP and Cu(II) or Fe(III). Cu(II)-dipeptide complexes such as Cu(Gly·Gly) reacted with H4DMP to give an intermediate complex Cu^{II}(Gly·Gly)(H4DMP) with the ESR parameters indicating the N(5) coordination. Stable intermediates are formed with Cu(Gly·Gly) which prefers a planar structure but not with Cu(bpy) (bpy = 2,2'-bipyridine). The pterin ring of oxidized pterin-6-carboxylate (PC) in Cu(Gly·Gly)(PC) is bound perpendicular to the coordination plane through N(5) with O(4) axially coordinated. N,O-coordination has also been detected for Fe-pterin complexes. These results indicate that the redox reaction takes place via Cu(II)- or Fe(III)-H4DMP binding and further suggest that at the active site of PAH the pterin cofactor is bound to the central metal ion prior to O_2 activation. Hydroxylation of phenylalanine ethylester with Cu(II) complex-H4DMP systems gave a mixture of o-, m-, and p-tyrosines.

On the other hand, investigations are being made on the preparation, characterization, and reactivity of Met92Gln and other mutant plastocyanins as substitutes for small metal complexes.

1) J. McCracken, S. O. Pember, S. J. Benkovic, J. J. Villafranca, R. J. Miller, and J. Peisach, *J. Am. Chem. Soc.*, **110**, 1069(1988).

2) Y. Funahashi, T. Kohzuma, A. Odani, and O. Yamauchi, *Chem. Lett.*, 385(1994).

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The H$_2$ uptake hydrogenase genes of *Azotobacter chroococcum*

The H$_2$-uptake hydrogenase (Hup) of *Azotobacter chroococcum* is a membrane bound, two subunit (α β) nickel-iron protein which oxidizes H$_2$ *via* the respiratory chain to produce ATP. The structural and processing genes coding for the synthesis and activity of these proteins, as well as those of similar hydrogenases from several other organisms, have been isolated and sequenced in recent years. Those from *A. chroococcum* occupy 15Kb of chromosomal DNA containing 16 contiguous open reading frames. In addition to this main *hup* gene region, we have isolated a second, separate region involved in the synthesis of protein(s) related to H$_2$-dependent respiration and to the level and stability of hydrogenase activity. Over 5Kb of this gene region has been sequenced and characterised by site-directed mutagenesis.

The activation of expression of the main *hup* gene region promoters has been investigated by introducing Tn5::*lacZ* constructs into the chromosomal *hup* genes. Our investigations suggest that these promoters are regulated *via* the redox state of the cell, possibly by a regulatory protein containing iron.

EPR Detection of Five-Coordinated Nitrosylhemoprotein in Denitrifying Bacteria

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Biological denitrification is a process which converts nitrate or nitrite to nitrous oxide or dinitrogen as follows. $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ Each reduction step is linked to the generation of ATP and all the reductases are known to be metalloenzymes and to be associated with metal-containing cofactors. It has been demonstrated that nitric oxide (NO) formed as an intermediate in the process is not released into the environment under physiological condition.

In the present work, we have measured the electron paramagnetic resonance spectra at frozen temperature for the cells of denitrifying bacteria to obtain information about the interaction of NO and metalloproteins in the intact cells. Denitrifying bacteria were cultivated in meat extract-peptone medium under denitrifying or non-denitrifying conditions. Among the bacteria, *Achromobacter xylosoxidans* NCIB 11015 was cultivated also in Mn-free synthetic medium under denitrifying condition. The characteristic three-line signal was observed in the whole cells of the bacteria under denitrifying conditions, but not under non-denitrifying conditions. The three-line signal was more distinctly detected in the cells of *A.xylosoxidans* NCIB 11015 cultured in Mn-free medium. This signal was quite similar to that of NO-adducts of cytochrome *c'* from *A.xylosoxidans* NCIB 11015[1] and that of five-coordinated nitrosyl heme model complexes. Therefore, this three-line signal could be assigned to nitrosylcytochrome *c'* containing a five-coordinated nitrosylheme.

[1]Yoshimura, T. et al., (1986) *Biochemistry* **25**, 2436-2442.

5,6-Dihydropyrimidinc Amidohydrolase, a Zinc Enzyme.

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5,6-Dihydropyrimidine amidohydrolase (EC 3.5.2.2)(DHPase) is the second enzyme in the pyrimidine degradative pathway catalyzing the reversible hydrolysis of 5,6-dihydrouracil (DHU) to N-carbamoyl-β-alanine. The enzyme was purified to homogeneity from bovine [1] and calf liver [2]. It consists of a tetramer with a molecular mass of 226 kDa, and contains four tightly bound zinc ions [1,2] which can be removed by chelators such as 1,10-phenanthroline [3]. The V/K for DHU measured either with the native zinc or the cadmium-substituted enzymes decreases at both low and high pH giving values of about 7.5-8 and 9-10. The lower pH value observed in V is perturbed significantly to lower pH (~6), and the high pK is not observed. The data are consistent with a general base mechanism and in addition suggest that the enzyme is present initially with water bound to the active site zinc. The enzymic general base with a pK of 7.5-8 is required to activate water for nucleophilic attack on C4 of DHU which is directly coordinated to the active site zinc. The second group with a pK of 9-10 likely reflects Zn-water ionization of the free enzyme. The water bound to the active site Zn is displaced by substrate binding, and thus the pK of 9-10 is not observed in the V profile. Other substrates of the amidohydrolase are hydantoin, phthalimide and succinimide. Glutaric acid monoamide, and 4-ureidobutyrate were found to be potent inhibitors of DHPase. NMR studies on [113]Cd-DHPase and spectral studies on Co- and Mn-DHPase are in progress.

[1] Lee, M.H., Crowling, R.A., Sander, E.G., and Pettigrew, D.W. (1986) Arch. Biochem. Biophys. 248, 368-378.

[2] Kautz, J., and Schnackerz, K.D. (1989) Eur. J. Biochem. 181,431-435.

[3] Jahnke, K., Podschun, B., Schnackerz, K.D., Kautz, J., and Cook, P.F. (1993) Biochemistry 32, 5160-5166.

Interaction of the cobalt with glutamate during the catalysis of coenzyme B$_{12}$-dependent glutamate mutase from *Clostridium cochlearium*

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Glutamate mutase catalyzes the reversibel adenosylcobalamin (coenzyme B$_{12}$)-dependent carbon skeleton rearrangement of (*S*)-glutamate to (2*S*, 3*S*)-methylaspartate. This is the first step in the glutamate fermentation pathway of a number of clostridia [1]. The enzyme generally consists of two protein components E (50 kDa) and S (15 kDa).

The genes encoding glutamate mutase from *Clostridium cochlearium* (*glmS* and *glmE*) were cloned, sequenced and overexpressed in *Escherichia coli* [2]. The deduced amino acid sequences of the protein components E (53553 Da) and S (14800 Da) show a high grade of identity to those from the corresponding genes from *C. tetanomorphum* [3]. Between the two genes a third gene named *glmL* is located. Although the function of GlmL is unknown, it can be grouped to the HSP70/actin/sugar kinase-superfamily [4] because of its ATP-binding site that was identified by computational alignments. The components of glutamate mutase, wich were overexpressed in *E. coli*, were purified with excellent yields.

Incubation of both components with coenzyme B$_{12}$ and (*S*)-glutamate generated an EPR-signal of higher spin-concentration and better resolution than those previously observed with the low amounts of enzyme which were available from *C. cochlearium* [5]. Due to its hyperfine splitting the signal is regarded as cob(II)amide with $g_{x/y}$ line about $g = 2.13$ and $g_z = 1.98$. After addition of the competitive inhibitors 2-methyleneglutarate or (2*S*, 4*S*)-4-fluoroglutamate, signals of the same type but with an altered line shape were observed suggesting interaction of the cobalt from coenzyme B$_{12}$ with the substrate or inhibitor. The extraordinary low g-values might also be ascribed to the coordination of an additional ligand to the cobalt as suggested for the rapid reaction intermediate of the B$_{12}$ dependent ribonucleotide reductase [6]. Addition of (*S*)-(2,3,4,4,-^{2}H$_5$)glutamate to the enzyme led to alterations of the superhyperfine-splitting especially of the z-line. Thus the sixth ligand of the cobalt was tentatively identified as one of the substrates hydrogens.

Over-night incubation of the enzyme with glutamate and coenzyme B$_{12}$ led to the formation of an inactive aggregate of both protein components and tightly bound B$_{12}$. The EPR spectrum showed that the substrate induced signal had disappeared and a 'classical' five fold coordinated cob(II)alamin has been formed ($g_x = 2.253$, $g_y = 2.225$, $g_z = 2.004$). This inactive glutamate mutase complex cannot only be produced *in vitro* but was also purified from *C. cochlearium* [5]. This supports the assumption that even *in vivo* glutamate mutase is inactivated after a number of turnovers. The occurence of this type of cob(II)alamin has always been accompanied with inactivation as it is discussed for the B$_{12}$-dependent 2-methyleneglutarate mutase [7] and ribonucleotide reductase [7].

[1]   Buckel, W. & Barker, H.A. (1974) J. Bacteriol. 117, 1248-1260
[2]   Zelder, O., Beatrix, B. & Buckel, W. (1994) FEMS Lett., in press
[3]   Holloway, D.E. & Marsh, E.N.G. (1993) FEBS Lett. 317, 44-48
[4]   Bork, P., Sander, C. & Valencia, A. (1992) Proc. Natl. Acad. Sci. 89, 7290-7294
[5]   Leutbecher, U., Albracht, S.P.J. & Buckel, W. (1992) FEBS Lett. 307, 144-146
[6]   Coffman, R.E., Ishikawa, Y., Blackley, R.L., Beinert, H. & Orme-Johnson, W. H. (1976) Biochim. Biophys. Acta 444, 307-318
[7]   Zelder, O. & Buckel, W. (1993) Biol. Chem. Hoppe-Seyler 374, 85-90
[8]   Pilbrow, J.R. & Winfield, M.E. (1973) Mol. Phys. 25, 1073-1092

µ-ALCOXO BRIDGED MANGANESE COMPLEXES OF 1,3-BIS(3-NO$_2$-SALICYLIDENE-AMINO)-2-PROPANOL AS MODELS OF BINUCLEAR ENZYMES. SYNTHESIS, MOLECULAR STRUCTURE AND MAGNETIC AND ELECTROCHEMICAL PROPERTIES.

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Most of the manganese dinuclear compounds relevant to biological sites involve simple mono- and/or bidentate organic molecules as ligands.[1] Our interest in polydentate Schiff bases stems from the fact that they can include a large variety of functions similar to those involved in the polypeptidic chains in which the multimetal centers of proteins or enzymes are embedded. In this contribution, we describe four distinct structural types for manganese complexes of the potentially pentadentate ligand 1,3-bis(3-NO$_2$-salicylideneamino)propan-2-ol (3-NO$_2$-salprOH$_3$), able to afford a variety of coordination modes related to the nature of its O N O N O donor set and the versatile accessibility of the dianionic "salprOH" or trianionic "salprO" forms. Mn^{II}(3-NO$_2$-salprOH) (**1**) is characterized by the MnL stoechiometry. Mn$^{II}_2$(3-NO$_2$-salprO)(AcO),4MeOH (**2**) is the first reported manganese(II) compound characterized by the Mn$_2$L stoechiometry with a ligand of this series. Two structurally different dinuclear Mn(III) complexes characterized by the same overall formulation (Mn^{III}(3-NO$_2$-salprO)(solvent))$_2$ can be obtained from the reaction of **1** with O$_2$, according to the coordinating ability of the solvent. In the asymmetric binuclear species (Mn^{III}(3-NO$_2$-salprO)(H$_2$O))$_2$ (**3**), one 3-NO$_2$-salprO pentadentate trianion bridges symmetrically the two manganese cations and the other ligand distributes asymmetrically its five donor atoms (O$^-$ N / O$^-$NO$^-$) between the two manganese cations. When **3** is dissolved in DMF, the water molecule is driven out of the manganese coordination sphere and a ligand environment reorganization affords [Mn^{III}(3-NO$_2$-SalprO)]$_2$,2DMF (**4**) in which the manganese(III) cations are bridged by the alcoholate anions of both 3-NO$_2$-salprO pentadentate trianions which distribute their four remaining donors symmetrically between both manganese cations. Besides the structural features and reactivity, the magnetic and electrochemical behaviour of these complexes will also be reported.

1) K. Wieghardt, *Angew. Chem., Int. Ed. Engl.*, **28**, 1153 (1989).

Methane as the Product of Reaction of Methyl-Coenzyme-M with Monovalent Nickel Complexes in Aqueous Solutions. A Model for the in vivo Activity of Cofactor F_{430}.

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Nickel enzymes are known to be prominent in the metabolism of anaerobic bacteria. The hydrocorphinoid nickel(II) complex - F_{430} is proposed to be involved in the last step of methanogenesis in methanogenic bacteria as a cofactor of methyl-coenzyme-M, MeCoM, reductase, the enzyme that catalyzes the reductive cleavage of MeCoM to coenzyme M and methane. The nickel is probably monovalent in the active form of the cofactor. It was decided to check whether model monovalent nickel complexes react with MeCoM in aqueous solutions to form methane?

The complexes 1 and 2 were reduced to the corresponding monovalent complexes and then reacted with MeCoM. The redox potentials of these complexes are -0.98 V and -1.25 V vs. SCE respectively which are similar to the redox potential of the Ni(II)/Ni(I) couple in native F_{430}, -0.89V vs. SCE.

1   2
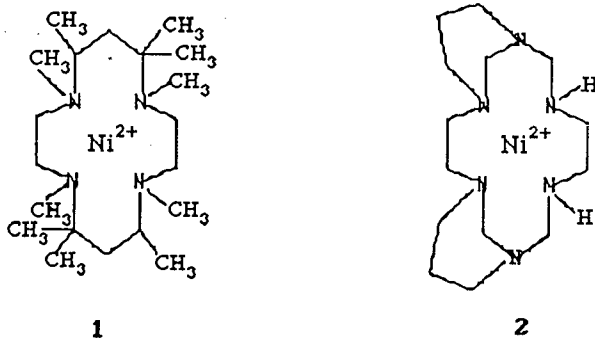
Solutions containing the monovalent nickel complexes 1 and 2 were prepared by irradiating with ionizing radiation He saturated solutions containing the divalent complexes and 0.01 M HCO_2Na. Deaerated solutions containing MeCoM were then injected into the vials containing the monovalent complexes. Vague traces of methane were detected at pH 7.4 while at pH 9.4 the yield of methane is over 10%. Blank experiments point out that MeCoM scavenges methyl free radicals via a mechanism which does not produce methane as the major product.

The results are in accord with the following mechanism for the methane production:

$$Ni(I)L^i{}_{aq} + CH_3SCH_2CH_2SO_3^- \longrightarrow Ni(II)L^i{}_{aq} + \cdot CH_3 + {}^-SCH_2CH_2SO_3^-$$

followed by:

$$Ni(I)L^i{}_{aq} + \cdot CH_3 \xrightarrow{H^+} L^i{}_{aq}Ni(II)\text{-}CH_3 \longrightarrow Ni(II)L^i{}_{aq} + CH_4$$
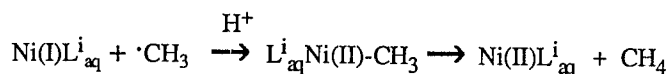
EPR and ENDOR-Investigations of Antiferromagnetically Coupled Dinuclear Manganese Complexes

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Exchange coupled manganese complexes containing at least a dinuclear core, are active sites in various enzymes, like the water oxidizing complex (WOC). Here we report recent EPR, ENDOR (electron nuclear double resonance), and ESEEM (electron spin echo envelope modulation) experiments on binuclear oxo–bridged dimanganese (III/IV) model complexes with different ligands. These complexes serve as models for the manganese complexes in the WOC of cyanobacteria and higher plants[1,2].

The ENDOR measurements were performed at very low temperatures using a home-built highly sensitive ENDOR resonator (1.8K - 300K temperature range)[3].

Simulations of EPR spectra taken at X– and Q–Band microwave frequencies give a well defined and consistent set of parameters for the g and manganese hyperfine tensors. All simulations were first performed for the Q–Band spectra, because the influence of second order effects due to the large manganese hyperfine couplings are smaller and the resolution of g anisotropy is higher in Q–Band. All parameters used for these simulations were kept constant for X– and Q–Band spectra of the different complexes.

X–Band ENDOR spectra were obtained for 3 types of dimanganese model complexes and also for the S_2 state (multiline signal) in the WOC[4]. An attempt is made to analyse the ENDOR spectra using the so called "point dipole" model. "Single-crystal-like" ENDOR spectra were taken on the highest field positions in the EPR spectrum. These spectra could be simulated using our program for orientationally selected ENDOR spectra. Selectively deuterated ligands helped in the assignment of the proton hyperfine couplings to specific nuclei in the complexes. The final analysis yielded a detailed picture of the electronic structure of the complexes and their surroundings. This is important for finally understanding the mechanism of the WOC in PS II and other related enzymes.

[1]K. Wieghardt, *Angew. Chem.* <u>101</u>, 1179(1989)

[2]U. Bossek et al. *J. Am. Chem. Soc.* <u>112</u>, 6387(1990)

[3]W.Zweygart, R.Thanner, W. Lubitz, *J. Mag. Res.* (1994) in press

[4]W. Zweygart, T. Weyhermüller, G. Renger, K. Wieghardt, and W. Lubitz, In *Research in Photosynthesis* , N. Murata, editor, Vol. 2, pages 289–292. Kluwer Academic Publishers. (1992).

Kinetic Analysis of Mn Peroxidase: The Reaction with Mn Complexes. I-Ching Kuan, Kenneth A. Johnson and Ming Tien, Department of Biochemistry and Molecular Biology and Center for Biomolecular Structure and Function, Pennsylvania State University, University Park, Pennsylvania 16802

Mn peroxidase from the lignin-degrading fungus *Phanerochaete chrysosporium* catalyzes the H_2O_2-dependent oxidation of Mn^{2+} to Mn^{3+}. Presteady-state methods were employed to characterize the reactions of free and chelated Mn^{2+} with the two-electron and one-electron oxidized forms of the enzyme, compound I and II, respectively. At pH 4.5, the optimum pH for steady state turnover, the reaction of compound I with Mn^{2+}, either free or complexed, is too rapid to measure by stopped flow methods. The reactions of compound I with Mn^{2+} can only be monitored under non-optimal conditions of pH 2.5. The reaction of compound II with Mn^{2+} is much slower than compound I. Chelators such as oxalate, lactate and malonate facilitated the reaction of Mn^{2+} with compound II. In contrast, succinate which does not readily form a complex with Mn^{2+}, and polyglutamate, which is polymeric, were ineffective in stimulating the reaction of Mn^{2+} with compound II. The 1:1 chelator/Mn^{2+} complex is the preferred substrate for compound II; this conclusion is based on known formation constants for the various Mn^{2+} complexes. The ubiquity of oxalate in wood degrading fungi would suggest that oxalate plays a major role in Mn peroxidase-dependent oxidation of lignin.

FINE-STRUCTURE FLUORESCENCE SPECTRA OF MODEL COMPOUNDS OF CYTOCHROME UNDER SELECTIVE LASER EXCITATION AT 4.2 K

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Site-selective spectroscopy makes it possible to obtain frequencies of molecular vibrations in the ground and first excited electronic states and may be used to investigate the structure and properties of cytochromes. In this case a cytochrome must be reconstituted with fluorescence chromofore. We have obtained the high-resolution fluorescence spectra of Zn(II) protoporphyrin IX (1), Zn(II) mesoporphyrin IX (2) and Zn(II) 2,3,7,8,12,18-hexamethyl-13,17-dipropylporphyrin (3) and their infrared spectra also. The set of investigated compounds make it possible to study manifestation of the vibrations of vinyl groups and propionic acid residues in vibronic spectra and vibrational interaction between porphyrin macrocycle and substituents.

The fluorescence spectra consist of more than 30 lines. According to the normal coordinate analysis of the molecules the substituents take part in the majority of the vibrations which are active in vibronic spectra. Among them the lines of 1 with vibrational frequencies 266, 296, 405, 414, 495, 599, 674, 695, 716, 921, 933, 971, 997, 1075, 1093, 1116, 1148, 1177, 1185, 1214, 1237 and 1431 cm^{-1}.The lines 405, 414 and possibly 997 cm^{-1}, which are absent in spectra of 2 and 3, were assigned to vinyl group vibrations. Propionic acid residues take part in the modes 373, 716, 997, 1075, 1177 and 1185 cm^{-1} of molecule 1. Assignment of vibrational frequencies obtained from vibronic, infrared as well as resonance Raman spectra was made on the base of normal coordinate analysis.

List of Contributors

*Oral presentation